Promoting Athlete Health and Performance:

The Role of Haemostatic Changes

in Response to Exercise

by

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Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

University of Tasmania

December 2017

Declaration of Originality

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Statement of Candidate and Co-Authorship Contribution of Jointly Published Work

The following people and institutions contributed to the publication or work undertaken as part of this thesis:

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11. Dr Andrew C Bulmer, Griffith University
12. Dr Shona L Halson, Australian Institute of Sport

Paper One: Validity of power settings of the Wahoo KICKR Power Trainer

- Located in Chapter Three.
- Candidate was the primary author, and contributed to 80% of the planning and the execution of the research project, with authors 1 and 2 also contributing to the conceptualisation of the research design. Author 5 provided the Wahoo KICKR Power Trainer for this study.
- The candidate and authors 1 and 4 assisted with data collection, whilst the candidate and authors 1, 2, and 4 significantly contributed to data analysis, whilst the candidate and authors 1, 2, 4 and 5 contributed to drafts of the manuscript.

Paper Two: The reliability of a laboratory-based 4km cycle time trial on a Wahoo KICKR Power Trainer

- Located in Chapter Four.
- Candidate was the primary author, and contributed to 90% of the conceptualisation of the research design, with authors 1 and 2 also contributing to the conceptualisation of the research design.
- The candidate completed all aspects of data collection individually.
- The candidate and authors 1 and 2 contributed to the analysis of data, whilst the candidate and all authors contributed to the drafts of the manuscript.

Paper Three: Time of day and short-duration high-intensity exercise influences on coagulation and fibrinolysis

- Located in Chapter Five.
- Candidate was the primary author, contributed to 80% of the study conceptualisation, in addition to contribution from authors 1, 2, 3 and 4.

- Candidate completed the majority of data collection, with assistance from authors 1, 2 and 4 with blood collection.
- Author 3 contributed significantly to the required laboratory work of the collected blood samples, teaching the candidate how to complete ELISA analysis.
- Authors 2 and 3 assisted the candidate and contributed significantly to statistical analysis and interpretation of the data.
- Candidate and authors 1, 2, 3 and 4 all contributed to the drafts and revisions of the manuscript.

Paper Four: Compression socks and the effects on coagulation and fibrinolytic activation during marathon running

- Located in Chapter Six.
- Candidate was the primary author, contributing to 80% of the planning of the research project, in addition to significant contributions from authors 1, 2, 3, 4, 9, 10 and 12, with author 1 playing a key role in the fostering of collaborations between the University of Tasmania, Griffith University and Queensland Hospital and Health Services.
- The candidate and authors 4, 6, 7, 8, and 11 played a key role in data collection, with authors 6, 7 and 11 contributing significantly to the processing of collected blood samples.
- The candidate and authors 3 and 4 contributed significantly to the execution of ELISA analysis on collected samples.

- The candidate and authors 1, 2, 3 and 4 all contributed significantly to the analysis of data, with the candidate and authors 1, 2, 3, 4, 6, 8, 9, 10, 11 and 12 significantly contributing to the drafts and revisions of the manuscript.

Paper Five: Reliability of power settings of the Wahoo KICKR Power Trainer after 60 hours of use

- Located in Appendix.
- Candidate was the primary author, contributing to 90% of the conceptualisation of the research project, with authors 1, 2 and 4 also contributing to the conceptualisation of the research project.
- The candidate and authors 1 and 4 assisted with the collection of data, with all authors significantly contributing to the interpretation and analysis of data and manuscript revision.

We, the undersigned, agree with the above stated "proportion of work undertaken" for each of the above published or submitted peer-reviewed manuscripts contributing to this thesis.

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Date: 3rd July 2018 4th July 2018

Statement of Contribution to Thesis

This thesis comprises of four research investigations, which have been completed almost entirely by the candidate, Emma Zadow (Sport Performance Optimisation Research Team, School of Health Sciences, University of Tasmania). The candidate played a leading role in the design of the studies, participant recruitment, data collection, data analysis and interpretation, and is first author on all manuscripts (chapters' three to six and appendix B). The following people and institutions also contributed to each of the studies (unless otherwise specified) as detailed:

- Associate Professor James W Fell (Sports Performance Optimisation Research Team, School of Health Sciences, University of Tasmania): Advised on study design, conceptualisation of study four, assisted with ethics applications, collection and statistical analysis of data and manuscript revisions.
- Dr Cecilia M Kitic (Sports Performance Optimisation Research Team, School of Health Sciences, University of Tasmania): Advised on study design, assisted with ethics applications and assisted with statistical analysis and manuscript revisions.
- Associate Professor Murray J Adams (School of Veterinary and Life Sciences, Murdoch University): Advised on study design, assisted with laboratory work (studies three and four), data analysis and manuscript revisions.
- Dr Sam SX Wu (Department of Health, Arts and Design, Swinburne University of Technology): Advised on study design, assisted with collection and statistical analysis of data and manuscript revisions.
- Associate Professor Stuart T Smith (Southern Cross University): study one: Assisted with provision of equipment and assisted with manuscript revisions.

- Associate Professor Indu Singh (Menzies Health Institute, Queensland and School of Medical Science, Griffith University): study four: Assisted with collection of data and manuscript revisions.
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- Ms Nerolie Bost (Department of Emergency Medicine, Gold Coast Health and School of Nursing and Midwifery, Griffith University): study four: Acted as a point of contact on the Gold Coast, assisted with manuscript revisions.
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- Associate Professor Julia Crilly (Department of Emergency Medicine, Gold Coast Health and School of Nursing and Midwifery, Griffith University): study four: Conceptualisation of study, advised on study design, sought ethics approval from Griffith University and Gold Coast Health, advised on manuscript revisions.
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- Dr Shona Halson (Senior Recovery Physiologist, Australian Institute of Sport): study four: Assisted with study design, provision of 2XU products and manuscript revisions.

The following students provided additional support on certain aspects of the thesis projects as follows:

- Sam Hughes, Myles Moore, Torben Partridge-Madsen, Taylor Wilczynski, Kahlia Perry, Marni Whish-Wilson, Georgia McCall, Sarah Mullen and Rachel Austin (School of Health Sciences, University of Tasmania), assisted with data collection and exercise testing (studies one, two and three), as part of their practicum work experience.

List of Publications

Most chapters of this thesis have been published in peer-reviewed journals. Accepted manuscripts that have been prepared throughout the duration of the PhD candidature are outlined below:

Zadow, E.K., Kitic, C.M., Wu, S.S.X., Smith, S.T. & Fell, J.W. "Validity of power settings of the Wahoo KICKR Power Trainer". *International Journal of Sports Physiology and Performance*, 2016, 11, 1115-1117.

Zadow, E.K., Fell, J.W. & Kitic, C.M. "The reliability of a laboratory-based 4km cycle time trial on a Wahoo KICKR Power Trainer". *Journal of Science and Cycling*, 2016, 5, 3, 23-27.

Zadow, E.K., Kitic, C.M., Wu, S.S.X. & Fell, J.W. "Reliability of power settings of the Wahoo KICKR Power Trainer after 60 hours of use". *International Journal of Sports Physiology and Performance. (Ahead of print:* https://doi.org/10.1123/ijspp.2016-0732; Accepted April 11, 2017).

Zadow, E.K., Kitic, C.M., Wu, S.S.X., Fell, J.W., & Adams, M.J. "Time of day and short-duration high-intensity exercise influences on coagulation and fibrinolysis". *European Journal of Sports Science*, Accepted December 15th, 2017.

Zadow, E.K., Adams, M.J., Wu, S.S.X., Kitic, C.M., Singh, I., Kundur, A., Bost, N., Johnston, A.N.B., Crilly, J., Bulmer, A.C., Halson, S.L., & Fell, J.W. "Compression socks and the effects on coagulation and fibrinolytic activation

during marathon running". *European Journal of Applied Physiology,* Accepted June 30th, 2018.

Sheahen, B.L., Fell, J.W., **Zadow, E.K.,** Hartley, T.F., Kitic, C.M. "Intestinal Damage Following Short Duration Exercise at the Same Relative Intensity is Similar in Temperate and Hot Environments". *Journal of Applied Physiology, Nutrition, and Metabolism,* Accepted May 25th, 2018.

Zadow, E.K., Adams, M.J., Kitic, C.M., Wu, S.X., Fell, J.W. "Acquired and Genetic Thrombotic Risk Factors in the Athlete". *Seminars in Thrombosis and Hemostasis,* Accepted July 19th, 2018.

Conference Presentations

Presentations arising from studies completed throughout the candidature.

June 2015, **European College of Sports Science, Malmo, Sweden;** Mini-Oral Presentation. *"Validity of the Wahoo KICKR Power Trainer and reliability of a 4km cycle time trial"*

July 2015, **Science and Cycling Conference, Utrecht, Amsterdam;** Oral Presentation. *"Validity of the Wahoo KICKR Power Trainer and reliability of a 4km cycle time trial"*

April 2016, **Research to Practice Exercise and Sports Science Australia (ESSA) Conference, Melbourne, Australia;** Poster Presentation. *"Sustained reliability of the Wahoo KICKR Power Trainer over an extended period of time"*

April 2016, **Research to Practice Exercise and Sports Science Australia (ESSA), Melbourne, Australia;** Oral Presentation. *"Validity of the Wahoo KICKR Power Trainer"*

July 2016, **European College of Sports Science, Vienna, Austria;** Oral Presentation. *"Time of day does not influence pacing and performance in a 4km cycling time trial"*

October 2016, **Sports Medicine Australia (SMA) Conference, Melbourne, Australia;** Oral Presentation. *"Time of day has no influence on performance and pacing during a 4km cycling time trial"*

January 2017, **Murdoch University, Western Australia, Australia**

Invited Seminar Presentation: *"Athlete health and performance: the role of haemostatic changes in response to exercise"*

June 2017, **Rural Health and Collaborative Research Symposium, Launceston,**

Australia; Oral Presentation. *"Haemostatic responses to exercise: time of day and short-duration high-intensity exercise effects on coagulation"*

October 2017, **Sports Medicine Australia (SMA) Conference, Langkawi, Malaysia;** Oral Presentation. *"Does time of day and short-duration high-intensity exercise effect haemostasis?"*

October 2017, **Haematology Society of Australia and New Zealand, the Australian and New Zealand Society of Blood Transfusion and the Thrombosis and Haemostasis Society of Australia and New Zealand Conference, Sydney, Australia**; Poster presentation**.** *"Does time of day and short-duration high-intensity exercise influence coagulation and fibrinolysis?"*

October 2017, **Haematology Society of Australia and New Zealand, the Australian and New Zealand Society of Blood Transfusion and the Thrombosis and Haemostasis Society of Australia and New Zealand Conference, Sydney, Australia**; Poster presentation**.** *"Does wearing compression socks during a marathon influence coagulation and fibrinolytic activation?"*

April 2018, **Exercise and Sports Science Australia Conference, Brisbane, Australia;** Three poster presentations. 1. *"Influence of travel, compression socks and marathon running on haemostatic activation"*. 2. *"Short-duration high-intensity exercise activates haemostasis regardless of time of day"*. 3. *"Compression socks reduce exercise-associated haemostatic activation in marathon running"*

Acknowledgements

First, I would like to express my most sincere gratitude to my amazing supervisors, Associate Professor James Fell, Dr Cecilia Kitic, Associate Professor Murray Adams and Dr Sam Wu- your expertise, encouragement, and unwavering support and patience throughout all aspects of my postgraduate career have been greatly appreciated. Thank you for taking the chance on a West Australian and converting me to a shorts-wearing-in-the-winter Tasmanian. I am extremely grateful to have benefited from your wisdom, your knowledge and most of all, your friendship.

I would also like to express my appreciation to the University of Tasmania, School of Health Sciences and the Sports Performance Optimisation Research Team, for providing me with the opportunities and facilities to complete my PhD. Without the funding of the Australian Institute of Sport, 2XU and Sports Medicine Australia, along with the assistance of some amazing students including: Mr Sam Hughes, Mr Torben Partridge-Madsen, Mr Myles Moore, Mr Taylor Wilczynski, Ms Kahlia Perry, Ms Rachel Austin, Ms Sarah Mullen, Ms Georgia McCall and Mrs Marni Whish-Wilson, I would not have been able to complete the studies required for my PhD. Thank you so much for all of your help, it was extremely appreciated. My deepest gratitude goes to my study participants who kindly gave so much time, blood, sweat and tolerance; this thesis would not have been possible without you.

Thank you to the members of the Sports Performance Optimisation Research Team, especially Sally McLaine and Katie-Jane (KJ) Brickwood (especially for Friday wine-day sanity), Glenys Holt, Dr Megan Thow, Dr Dana Lis, Dr Daniel Zuj, Nathan Lawler and Nicole Gordon, for your genuine friendship, support, guidance and PhD meme tagging throughout this incredible journey. I could not have asked for a better group to share this experience with.

I would also like to thank my family, the Zadow's, the Heinrich's, and the Waghela's for all of your encouragement and support, whether it be monetary or emotional, throughout the duration of my PhD. I look forward to seeing what the future holds and know I can rely on your continuous support.

General Abstract

Background

Athletes are frequently exposed to training- and competition-induced risk factors that increase the risk of injury and illness, including venous thromboembolism (VTE). Whilst VTE is frequently associated with physical inactivity and non-athletic populations, approximately 1 in 1000 athletes will experience a post-exercise thrombotic episode, similar to that of the general population.

Aims

The overarching aim of the series of studies completed in this thesis was to investigate factors that may influence the overall haemostatic response, as demonstrated by activation of the coagulation and fibrinolytic systems in well-trained athletes. To investigate this aim, four studies were undertaken. The first study aimed to examine the validity of a new cycling ergometer, the Wahoo KICKR Power Trainer (KICKR). The second study aimed to investigate the reliability of a performance test, a 4km cycling time trial (TT). Once the ergometer was validated and TT performance test deemed to be reliable, a third study used both to investigate the influence of time of day on coagulation responses to a short-duration high-intensity bout of exercise, whilst investigating the potential existence of diurnal rhythms within markers of coagulation in well-trained cyclists. The final study aimed to investigate if sports compression clothing when worn during a longer bout of exercise could influence the haemostatic responses to exercise.

Results

Study One / Study Two: The KICKR displayed accurate measurements of power between 250 and 700Watts (W) at cadences of 80-120revolutions per minute (rpm),

with an ergometer error/bias of -1.1% (95% Limits of Agreement (LoA): -3.6% to 1.4%). Average power was shown to be highly reliable within the 4km cycling TT, with an average Intraclass Correlation Coefficient (ICC) and typical error of measurement (CV) of 0.94 (95% Confidence Intervals [CI]: 0.85-0.98) and 3.4% (95%CI: 2.7-4.7%) respectively. These results indicate the KICKR is a suitable ergometer and a 4km TT a suitable performance test for the completion of further studies utilising well-trained cyclists.

Study Three: A 4km TT was shown to significantly increase plasma concentrations of tissue factor (TF: $p<0.0005$), tissue factor pathway inhibitor (TFPI: $p<0.0006$), thrombin anti-thrombin complexes (TAT: $p<0.0012$) and D-Dimer ($p<0.0003$), regardless of the time of day the exercise was performed. A time of day response was observed in pre-exercise TF ($p=0.004$) and TFPI ($p=0.031$), with 0830 h higher than 1730 h ($p<0.001$), whilst levels at 1730 h were less than those at 2030 h ($p=0.008$). However, no significant effects for time of day for TAT ($p=0.364$) and D-Dimer ($p=0.228$) were reported.

Study Four: When worn during a marathon, compression socks (SOCKS) significantly attenuated the post-exercise increase in D-Dimer compared to the control group (median (range) SOCK: +9.02, (-0.34 to 60.7) ng/mL, CONTROL: +25.48, (0.95 to 73.24) ng/mL, $p=0.008$). TF was increased following the marathon run (median (range), SOCK: +1.19, (-7.47 to 9.11) pg/mL, CONTROL: +3.47, (-5.01 to 38.56) pg/mL, $p=0.001$), but there was no significant difference between the compression and control groups. No significant post-exercise changes were observed for TAT and TFPI ($p>0.05$).

Conclusions

The major conclusions of this thesis are:

1. The KICKR is a valid ergometer for measuring cycling power output.
2. A 4km cycling TT, when completed on the KICKR, is a reliable performance test for well-trained cyclists.
3. A short-duration high-intensity TT significantly activates the coagulation and fibrinolytic systems in well-trained cyclists, regardless of the time of day the TT is performed.
4. TF and TFPI are influenced by time of day, suggesting an increased potential for coagulation activation in the morning.
5. When worn during a marathon, compression socks reduce exercise-associated fibrinolytic activity as reflected by lower D-Dimer concentrations.

The significance of this series of studies is that they have demonstrated the duration of exercise and the time of day exercise is performed influence coagulation responses within a well-trained population. In addition, this thesis contributes to the scarce literature on the use of compression garments and haemostatic responses in endurance based exercise, establishing that the use of sports compression socks may be beneficial in the prevention of VTE. The findings of this thesis may assist coaches, sports scientists and the athletes themselves to consider preventative measures for VTE, especially in athletes who are genetically predisposed to hypercoagulability.

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Common Abbreviations

Selected abbreviations throughout the text

aPTT	activated partial thromboplastin time
AROM	active range of motion
AST	aspartate transminase
Bla	blood lactate
bpm	beats per minute
BW	body weight
CALRIG	calibration rig
CG	compression garments
CI	confidence intervals
CK	creatine kinase
CMJ	counter movement jumps
CV	coefficient of variation
DOMS	delayed onset of muscle soreness
DVT	deep vein thrombosis
ELISA	enzyme-linked immunosorbent assay
ES	effect size
F	female
F 1+2	prothrombin fragment 1+2
FPA	fibrinopeptide A
FV	factor five
FVII	factor seven
FVIII	factor eight

FVL	factor five leiden mutation
FIX	factor nine
FX	factor ten
FXII	factor twelve
h	hour
Hct	haematocrit
Hgb	haemoglobin
HR	heart rate
ICC	intraclass correlation coefficient
KICKR	Wahoo KICKR Power Trainer
km	kilometre
LDH	lactate dehydrogenase
LoA	limits of agreement
m	metres
M	males
min	minutes
mmHg	millimetres of mercury
ng/mL	nanograms per millilitre
PAI-1	plasminogen activator inhibitor
PC	protein C
PS	protein S
pg/mL	pictograms per millilitre
PLT	platelets
PTT	partial thromboplastin time
RBC	red blood cell

RM	repetition maximum
RPE	rating of perceived exertion
RPM	revolutions per minute
s	seconds
SCN	suprachiasmatic nucleus
submax	submaximal
TAT	thrombin anti-thrombin complexes
TF	tissue factor
TFPI	tissue factor pathway inhibitor
t-PA	tissue plasminogen activator
TT	time trial
TTE	total time to exhaustion
µg/L	microgram per litre
V_E	ventilation
VO_{2max}	maximal oxygen uptake
VT	ventilatory threshold
VTE	venous thromboembolism
W	watts

Infrequent abbreviations used through the thesis are defined *in situ.*

Chapter One: Thesis Introduction and Overview

1.1 Thesis Organisation

This doctoral thesis contains a series of four studies aimed at investigating factors that may influence the overall haemostatic responses in well-trained athletes.

Chapter One: Contains a general introduction of the factors comprising the themes of this thesis, identifying the individual aims and significance of the research, in addition to the general layout of the thesis.

Chapter Two: Comprises a review of the literature, providing background information supporting the research studies of this doctoral thesis.

Chapter Three: Contains study one of this thesis, which involved the investigation of the validity of power output generated by a cycling ergometer, the Wahoo KICKR Power Trainer, in comparison to power generated by a dynamic calibration rig, over power outputs and cadences typically observed within cycling (100-999W at cadences of 80-120rpm).

Chapter Four: Contains study two of this thesis. Once the KICKR was shown to provide valid measures of power output, study two investigated the reliability of a 4km cycling TT using well-trained cyclists over three separate occasions, when performed on the Wahoo KICKR Power Trainer.

Chapter Five: Contains study three of this thesis. Once deemed suitable, the KICKR and a 4km cycling TT was used in study three to investigate the influence of time of day on the coagulation and fibrinolytic responses to exercise in fifteen well-trained cyclists, over five separate time points (0830, 1130, 1430, 1730 and 2030 h). In addition, potential diurnal rhythms in resting markers of coagulation and fibrinolysis were investigated.

Chapter Six: The fourth and final study of this thesis aimed to investigate if compression socks attenuated haemostatic responses when worn during a marathon run. Sixty-seven participants (43 males and 24 females) were allocated into a compression or control (no socks) group and completed the 2016 Gold Coast Marathon, with pre- and post-exercise venous blood samples collected and analysed for coagulation and fibrinolytic activation.

Chapter Seven: The discussion chapter comments on the major findings of this thesis, and discusses the potential implications of the research undertaken, providing directions for future research and an overall conclusion.

1.2 Background

Athletes are frequently portrayed as the epitome of health and well-being due to their high level of physical fitness [1]. To maintain their competitive and elite-level status, athletes are constantly exposed to training- and competition-induced thrombogenic risk factors including injury and inflammation, dehydration and haemoconcentration, and long-haul travel [2-5]. The combination of these risk factors could lead to the development of a venous thromboembolisms (VTE), however, as VTE is rare within the athletic population, VTE is often misdiagnosed and poorly treated, leading to death in the worst case scenario [2, 6]. Indeed, investigations into fatalities in 243 football players revealed that five deaths resulted from VTE [6], with four of these cases precipitated by a traumatic injury and surgery. However, known risk factors for VTE have previously been examined in untrained, healthy populations [7-9], which may not be applicable to an athletic population. Furthermore, there are methodological questions not addressed by these studies, including the duration and intensity of the exercise, and the time of the day that exercise is completed, making interpretation of findings difficult.

1.2.1 Haemostasis, Exercise and Compression

Exercise has considerable effects upon haemostasis, with transient increases in blood coagulation [8, 10, 11], platelet aggregation [12] and fibrinolytic activity [8, 13, 14], with the degree of activation dependent on the duration and intensity of the exercise bout, the study population investigated and the time of day the exercise is performed [9, 15-17]. Whilst activation of the coagulation system has been reported in extremely short (15-90 s) [18] and longer (>20 min) [19] duration exercise bouts as indicated by significant increases in thrombin anti-thrombin (TAT) complexes and prothrombin fragment 1 + 2 (F 1+2), consensus is lacking with regards to exercise bouts between 90 s to 20 min. Many sporting events such as cycling time trials are completed within this duration of time, however the response of the coagulation system during this period is yet to be extensively investigated.

In longer-duration, low to moderate intensity exercise (i.e. marathon running, triathlons and ultramarathons), multiple studies report significant activation of the coagulation and fibrinolytic systems [10, 20, 21]. With VTE associated with a simultaneous increase in coagulation activation and a decrease in fibrinolysis, disrupting the dynamic haemostatic balance, research on how to prevent or lower this coagulation activation, within and immediately following exercise, is relatively scarce. Due to the proposed benefits of compression garments used in the treatment and prevention of lower extremity pathologies including deep vein thrombosis (DVT) within a clinical population [22, 23], it has been suggested that similar outcomes may be observed when combined with exercise, despite a lack of clear scientific evidence of their effects on physiology. Whether compression garments influence haemostatic activation during endurance exercise is yet to be extensively investigated. To date, only one study has examined the efficacy of compression socks on coagulation

responses during a marathon run. The authors reported an overall decrease in haemostatic activation when compression socks were worn [24].

1.2.2 Haemostasis, Time of Day and Exercise

As well as exercise duration and intensity, the time of day exercise is completed may play an essential role in the degree of coagulation and fibrinolytic activation. Markers of coagulation and fibrinolysis have been demonstrated to possess circadian rhythms, with hypercoagulability, hypofibrinolysis and increased platelet aggregation observed to occur between 0600 and 1200 h [7, 25-27], coinciding with an increase in the frequency of thromboembolic events [28, 29]. Therefore, it has been suggested that the safest time of day to exercise to prevent the incidence of VTE is the afternoon (1200 h onwards), whilst all morning exercise should be avoided completely (0600-1200 h) [30]. The time of day in which an athlete exercises is often unavoidable, as they are required to compete and train over the course of a 24-hour day based upon the scheduling of training and competition, with this suggestion consequently unrealistic in elite and well-trained athletes. For this reason, research investigating the influence of exercising at different times of the day on haemostatic responses in an athletic population are warranted.

1.2.3 Conclusion

Given the limited research on haemostatic responses to exercise within an athletic population [9, 19, 31], the need to investigate “athlete-specific” risk factors utilising an athletic population is therefore required. To date, there is scarce evidence of coagulation and fibrinolytic responses to an exercise bout between 90 s to 20 min of duration, with this duration of exercise frequently employed in most training and competition requirements. Subsequently, with markers of coagulation demonstrated to possess their own circadian rhythms [25, 26] and with exercise well-documented to

activate both the coagulation and fibrinolytic systems [20, 21], it remains unclear if exercising at different times of the day may influence the degree in which these systems are activated, especially within an athletic population. Despite a lack of clear scientific evidence of their effects on physiology within sporting performance and recovery, compression garments are frequently used within a clinical setting for the prevention and treatment of lower extremity pathologies [23]. Therefore, the use of compression garments, when implemented within a sporting environment, may have the potential to reduce the risk of VTE [24]. Whether compression garments favourably influence haemostatic responses within exercise remains relatively unknown, highlighting the need for further research within this field. Subsequently, the primary purpose of this thesis was to investigate factors that have the potential to influence overall haemostatic responses in well-trained athletes.

1.3 Significance of the Research

In general, athletes have a low risk of VTE, occurring at a rate of ~ 1 in 1000 people [32, 33], similar to that of the general population. In general, athletes have a low risk of VTE; however, this population is exposed to many acquired thrombogenic risk factors as a direct result of training and competition [32, 34] which can negatively impact overall performance outcomes. Furthermore, diagnosis of VTE within an athletic population is often overlooked with symptoms of VTE often mistaken for general training soreness and sporting injuries, which in the worst scenario, can result in death [6, 35]. Whilst numerous “athlete-specific” risk factors have been identified, these investigations typically employ “apparently-healthy” populations only, with these findings difficult to interpret and apply to an athletic population. With extremely short-duration and high-intensity exercise frequently employed by athletes regardless of the sport completed, the coagulation and fibrinolytic responses within this

population are poorly understood. Therefore, the overall aim of this thesis was to contribute to the lack of research on haemostatic responses within a well-trained population, especially with regards to short-duration high-intensity exercise. Additionally, the influence of exercising at different times of day on coagulation responses may be of importance for athletic populations, especially in athletes predisposed to hypercoagulability, with athletes required to train and compete at different times of the day. The findings from this thesis may inform guidelines for scheduling of training on an individualised basis. With a surge in popularity of endurance events (including marathons, triathlons and ultramarathons), the potential for VTE is significantly increased. Therefore, the ability to minimise the potential risk for thrombosis formation is of high priority. The use of compression garments is potentially a cost effective, preventative measure of VTE during and following exercise. While compression garments are used for the enhancement of sporting performance and recovery, the influence of compression garments on haemostatic responses to exercise has not been extensively investigated. Whilst widely employed in a clinical setting in the prevention of lower-limb pathologies including DVT, wearing compression garments whilst exercising has been suggested to reduce the overall risk of VTE. The findings of this research may highlight the beneficial effects of compression garments on haemostatic responses whilst undertaking endurance exercise.

1.4 Research Aims

The overarching aim of the series of four studies was to investigate factors that may influence haemostatic responses in well-trained athletes. The first two studies determined the validity and reliability of a relatively new cycling ergometer, the Wahoo KICKR Power Trainer (KICKR) required for a performance test and a 4km

cycling time trial (TT), to be used in subsequent studies. Once validated and proven to be reliable, the KICKR and the 4km TT was used in study three to: 1. Investigate the influence of time of day on coagulation responses to a shorter-duration high-intensity bout of exercise, and 2. Investigate the potential existence of diurnal rhythms within markers of coagulation over five individual time points. The fourth and final study then investigated the effect of sports compression clothing (i.e. compression socks), when worn during endurance exercise on the haemostatic response to exercise. The specific aims of each study are presented below.

Study One and Study Two: To enable the investigation of coagulation in athletes, study one aimed to determine the validity of a cycling ergometer and study two examined the reliability of a performance test.

1. Does the Wahoo KICKR Power Trainer (KICKR) provide valid measures of power output?

2. Is a 4km cycling time trial (TT) reliable when completed on the KICKR?

Study Three: The KICKR and a 4km cycling TT was then used to investigate the influence of time of day on the coagulation responses to exercise. In addition, the potential diurnal rhythms in markers of coagulation were investigated.

1. When completed by well-trained cyclists, will a short-duration high-intensity bout of exercise (4km cycling TT) influence the coagulation and fibrinolytic systems? In addition, will these responses vary when exercising at different times of the day (0830, 1130, 1430, 1730 and 2030 h)?

2. Is a time of day response/ diurnal rhythm present within thrombin anti-thrombin complexes (TAT), tissue factor (TF), tissue factor pathway inhibitor

(TFPI) and D-Dimer, markers associated with the coagulation and fibrinolytic systems?

Study Four: To investigate if compression socks attenuates haemostatic responses, study four aimed to examine the influence of compression socks on coagulation and fibrinolysis when worn during a marathon run.

1. When worn during a marathon (42.2km), can compression socks alter the overall haemostatic response?

Chapter Two: Literature Review

2.1 Introduction

Venous thromboembolism (a collective term for DVT: a blood clot located in the deep veins) and/or pulmonary embolism (a blood clot located in the lungs)) [36], is frequently associated with physical inactivity and a sedentary lifestyle. Significantly, several case studies of high-level and elite-level athletes developing a VTE have been reported [2, 5, 37, 38]. While exercise is associated with numerous health benefits [39-42], exercise disrupts haemostasis, and when combined with other thrombogenic risk factors, increases the risk of VTE. This may result from a temporary increase in the activation of coagulation [8, 11, 43] immediately upon exercise completion [20], with a subsequent decrease in fibrinolytic activity. Although exercise-induced disturbances in the haemostatic balance may not be detrimental to most participants, approximately 1 in 1000 athletes will experience a post-exercise thromboembolic incidence, similar to that of the general population albeit in the absence of exercise [44].

While athletes are considered to be healthy due to their high levels of physical fitness, they are frequently exposed to acquired individual-specific risk factors associated with hypercoagulability, including long-haul travel, dehydration, post-exercise haemoconcentration, and polycythaemia [3]. Furthermore, athletes are exposed to "athlete-specific" risk factors including exercise (duration and intensity), trauma, and the time of day at which exercise is performed [7, 31, 45], potentially enhancing the risk for VTE. When these factors are considered in conjunction with genetic pre-dispositions to hypercoagulability present in some athletes, an overall increased risk for VTE is present [32, 46, 47]. Furthermore, diagnosis of DVT within an athletic population is often overlooked with symptoms of a DVT (i.e. pain, tenderness and swelling), often mistaken for general training soreness and sporting injuries.

To date, the majority of research examining the mechanism of coagulation activation (as measured by *in-vivo* markers of thrombin generation (i.e. TAT and F 1+2) following exercise, have only used "apparently-healthy" and untrained populations [16, 18, 48, 49]**.** Indeed, the majority of these studies have failed to report factors that may influence overall outcomes, including the time of day that exercise is completed. Therefore, studies investigating coagulation responses to exercise in well-trained athletic populations when completed at different times of the day are clearly required. In addition, conflicting responses of post-exercise coagulation activation have been observed in studies of varying duration and intensities [14, 49], with several studies demonstrating post-exercise activation [11, 19, 20, 50], whilst others have failed to demonstrate this activation of the coagulation system [51, 52]. Thus, the conflicting responses of coagulation activation with varying durations and intensities highlights the need for further investigation, especially within a well-trained and athletic population. This chapter therefore aims to identify significant gaps within the literature, whilst critiquing and discussing factors that may influence haemostasis in an athletic population.

2.2 Overview of Haemostasis

Haemostasis is the physiological process that maintains the balance between excessive bleeding and clotting, maintaining normal blood circulation [53-56]. This mechanism is continuously active at low levels and is achieved through a dynamic equilibrium between activators and inhibitors of haemostasis, including, 1) the vascular system, 2) blood platelets, 3) the coagulation system, 4) physiological inhibitors of coagulation, and 5) the fibrinolytic system [9, 53, 57].

2.3 Overview of Virchow's Triad

Virchow's Triad comprises three factors that broadly contribute to the development of thrombosis. These were first postulated, then formally described by Rudolf Virchow in the mid-19th century [1]. Virchow's Triad was initially proposed for the development of VTE, however the same three factors contribute to the development of arterial thrombosis and disseminated intravascular coagulation [58]. The factors of Virchow's Triad favourable to VTE consist, of 1) hypercoagulability, 2) vessel wall injury, and 3) stasis [1, 57, 59] (**Figure 2.1**). In addition to these factors, activation of coagulation may arise from acquired or hereditary risk factors (**Table 2.1**). With haemostasis constantly active at low levels, changes to one or more factors associated with Virchow's Triad may contribute to the increased risk of thrombosis [53].

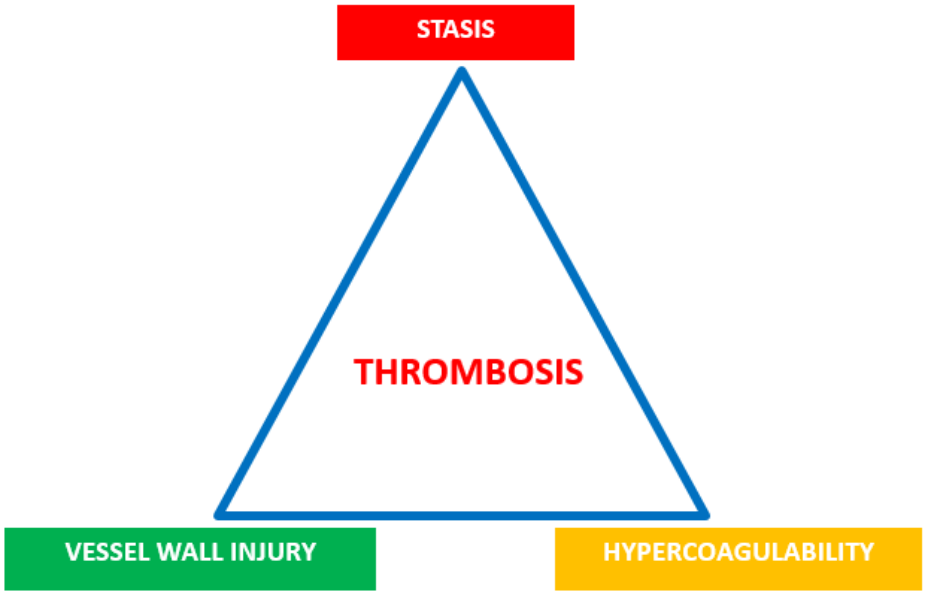


Figure 2. 1 *Illustration of Virchow's Triad, highlighting the three factors contributing to thrombosis development.*

Table 2.1 *Acquired and inherited risk factors demonstrated to pre-dispose individuals to venous thromboembolism [60].*

Acquired	Age Pregnancy Hormonal changes (oral contraceptive/ hormone replacement therapy) Obesity Injury and immobilisation Surgery Prior VTE Varicose veins	
Inherited		**Frequency (%) of thrombophiliac syndromes (general population)**
	Protein C deficiency	0.14-0.17
	Protein S deficiency	Not specified
	Anti-thrombin deficiency	0.02-0.17
	Factor V Leiden mutation	4-6
	Prothrombin (G20210A) gene mutation	1.7-3.0
	Activated Protein C resistance	3.6-6.0

2.4 Major Components of Haemostasis

2.4.1 Vascular System (Endothelium)

The vascular system, lined by endothelial cells, plays a pivotal role in the regulation of haemostatic balance by providing an interface between tissues and the blood [27, 55]. Endothelial cells are involved in each of the major haemostatic pathways [27, 55, 57], helping to prevent thrombosis by various anticoagulant and antiplatelet mechanisms (**Figure 2.2**) [27, 55-57]. They do this by maintaining blood viscosity via vasodilation, through the release of endothelium derived relaxing factor (nitric oxide) and prostacyclin (PGI_2), inhibiting adhesion and activation of blood platelets, inhibiting coagulation, and triggering fibrinolysis [27, 55, 57] .

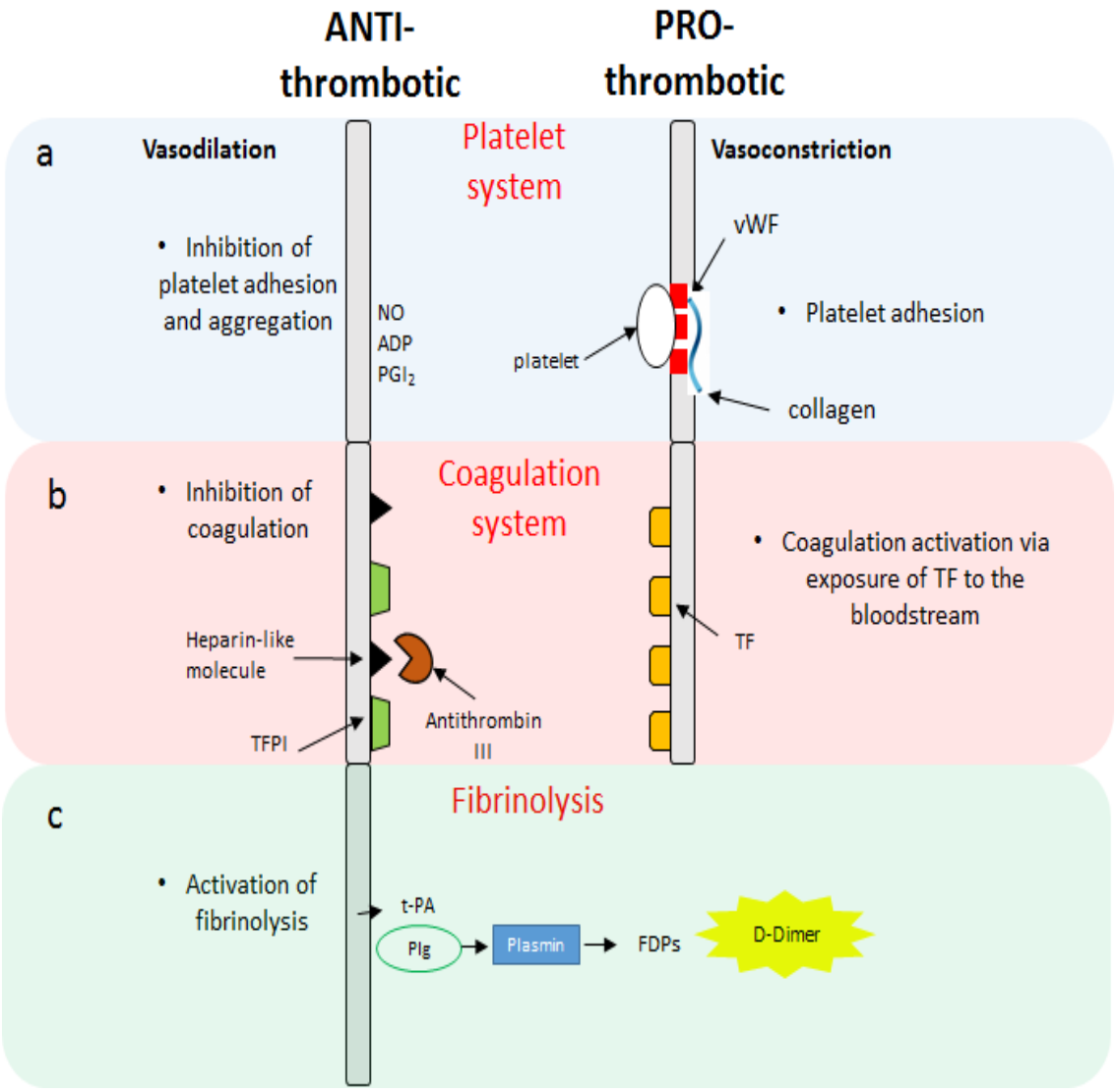


Figure 2.2 *Schematic representation of the role played by the vascular endothelium in the platelet, coagulation and fibrinolytic systems. a) Platelet mediated haemostasis is triggered via the exposure of the sub-endothelial to the blood. In response, vWF initiates the adhesion of platelets to the sub-endothelium, acting as a bridge between tissue and platelets, binding to collagen [55]. b) The exposure of TF to the blood stream initiates activation of the coagulation system through a cascade of events, resulting in the generation of thrombin. The natural anticoagulant TFPI and the main inhibitor of thrombin, anti-thrombin III, help to regulate thrombin generation. c) t-PA is produced by vascular endothelial cells and is released into the blood stream following vascular damage, initiating the activation of fibrinolysis. t-PA activates plasminogen forming plasmin, with plasmin then digesting fibrin, resulting in the production of fibrin degradation products and D-Dimer [61].* ***Abbreviations:*** *NO nitric oxide; ADP adenosine diphosphate; vWF von Willebrand Factor; TF tissue factor; TFPI tissue factor pathway inhibitor; t-PA tissue plasminogen activator; Plg plasminogen;* PGI_2 *prostacyclin.*

2.4.2 Platelets

Blood platelets are the first responders upon damage to the vascular endothelium. The exposure of platelet activating substances, i.e. collagen to the bloodstream, promotes the adherence of platelets to the damaged site, with von Willebrand factor (vWF) mediating the initial adhesion of platelets (haemostatic plug) to the sub-endothelium [54, 55]. Once adhered, they undergo activation, releasing agonists such as thromboxane A2, and adenosine diphosphate, inducing vasoconstriction and resulting in the recruitment of other platelets to the site of vessel injury [54, 55, 62]. The activation of the platelet system results in simultaneous activation of the coagulation system and is discussed in further detail below (*2.4.3. Blood Coagulation: the Cell-Based Model of Coagulation).*

2.4.3 Blood Coagulation: The Cell-Based Model of Coagulation

Blood coagulation comprises a complex series of interactions between proteases and cofactors that are present within the circulation [54, 57]. Historically, the two main pathways, known as the intrinsic (all components present within the blood) and extrinsic (exogenous factors required) pathways, were activated by foreign surface contact via factor XII (FXII) and the release of tissue factor (TF) from endothelial cells, respectively [54]. Through either pathway, coagulation converged at the common pathway with factor X (FX), resulting in the FXa:FVa (prothrombinase) complex and the formation of low amounts of thrombin [40, 52, 63]. From this historical model the extrinsic and intrinsic pathways have formed the basis of diagnostic coagulation screening tests, still used by clinicians to screen for abnormalities of haemostasis [64].

More recently, it has been proposed by Monroe and Hoffman [63] and described in detail by Versteeg et al. [54], that haemostasis occurs in three distinct, but overlapping phases, termed the cell-based model of coagulation. These include, i) *initiation*, where low amounts of TF are produced on cells such as monocytes and endothelial cells, ii) *amplification*, where platelets provide a pro-coagulant surface for increased coagulation and thrombin generation, and iii) *propagation*, where large amounts of thrombin are generated on the platelet surface, resulting in fibrin-rich blood clots (**Figure 2.3**).

In this cell-based model, the initiation of coagulation (also termed the TF pathway) begins via disruption or damage to the vasculature, exposing TF to the bloodstream [56] (**Figure 2.3**). TF binds coagulation FVII, promoting proteolysis and activation of FVII to FVIIa, resulting in the formation of the TF:FVIIa complex [54]. TF:FVIIa proteolytically cleaves traces of FIX and FX into FIXa and FXa, respectively. FXa and FVa form a prothrombinase complex on TF-expressing cells, resulting in the generation of thrombin [54, 57, 63].

The amplification phase of coagulation results in the movement from a TF-bearing cell to the platelet surface, enhancing coagulation activation [63]. Platelet adherence to extravascular matrix components at the site of vascular injury are established [54, 55], resulting in platelet activation, whilst localising platelets to the primary site of TF exposure. This close proximity to thrombin generated by TF-bearing cells amplifies the pro-coagulant signal, enhancing platelet adhesion [65] and activating FV, FVIII and FXI [66]. With the activation of platelets, and cofactors V and VIII bound to their surfaces, thrombin generation is enhanced [63] (**Figure 2.3**).

The final phase of coagulation generates large amounts of thrombin that contributes to the formation of a blood clot. It is during this phase that activated proteases combine with their cofactors on the platelet surface, with continuous generation of thrombin [63]. Platelets express high affinity receptors for activated FIX [67], FX [68] and FXI [69]. When FIX is activated to FIXa, it forms a complex with FVIII (FVIIIa:FIXa) [70]. This complex is crucial for haemostasis and has been shown to activate FX on the platelet surface, resulting in FXa. As described by Hoffman and Monroe [63], FXa then moves directly into a complex with activated FVa. This FXa:FVa complex is then capable of producing the thrombin required to form a fibrin rich blood clot.

To ensure the haemostatic balance is maintained, excess fibrin formation is limited by numerous anticoagulant mechanisms, including tissue factor pathway inhibitor (TFPI) [57] (*see sections 2.4.3(2) and 2.4.3(4)).* Furthermore, the fibrinolytic pathway is activated simultaneously with blood coagulation to prevent excessive clotting [41, 57].

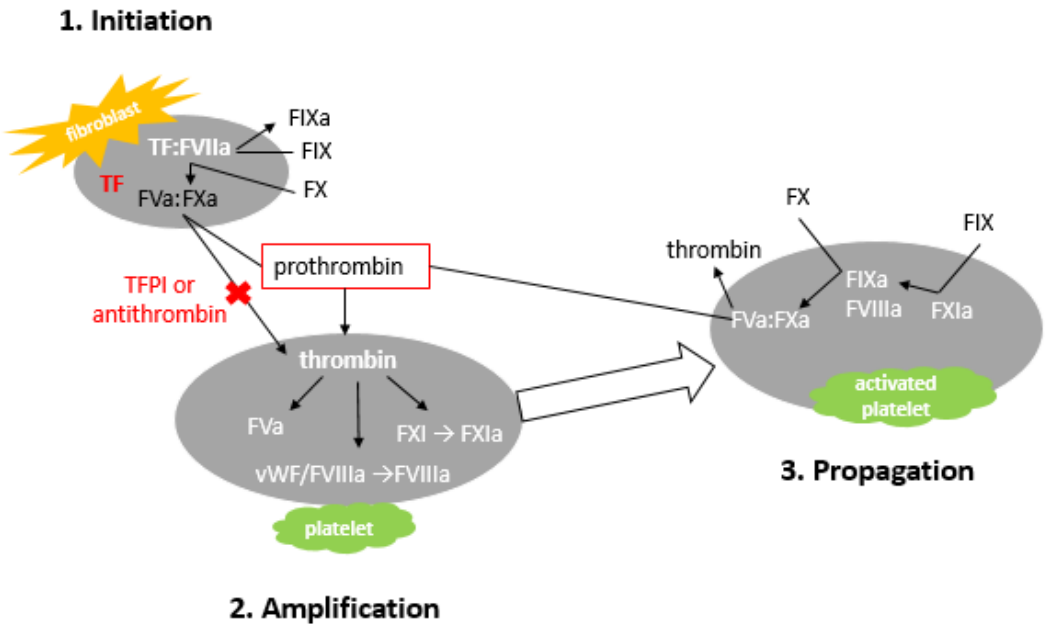


Figure 2.3 *Schematic representation of the cell-based model of coagulation. 1) The initiation of coagulation takes place on TF-bearing cells. The TF:FVIIa complex activates FIX, FX. FXa then combines with FVa forming the prothrombinase complexes on TF-bearing cells [66]. FXa that dissociates from TF-bearing cell is rapidly inhibited by TFPI or anti-thrombin. 2) Amplification requires the "action" to move from TF-bearing cells to the platelet surface. Thrombin generated on TF-bearing cells amplify the initial pro-coagulant signal, activating FV, FVIII and FXI [65]. vWF /FVIII binds to platelets and is cleaved by thrombin to activate FVIII and release it from vWF [71]. 3) Propagation phase occurs on activated platelets. The tenase (FVIIIa:FIXa) and prothrombinase complexes (FVa:FXa) are assembled on the platelet surface and thrombin generation occurs, resulting in fibrin polymerisation [63]. Schematic redrawn from Monroe and Hoffman [63].* ***Abbreviations:*** *TF tissue factor, TFPI tissue factor pathway inhibitor, vWF von Willebrand Factor.*

2.4.3(1) Tissue Factor

TF is a transmembrane glycoprotein expressed constitutively on cell surfaces, including fibroblasts and pericytes in, and surrounding blood vessel walls and epithelial cells [54, 57, 72]. TF is the primary physiological initiator of coagulation, achieved via a break in the vascular wall allowing plasma to come into direct contact with TF-bearing extravascular cells [73, 74]. In addition, monocytes and smooth muscle cells can be stimulated to produce TF by cytokines and other inflammatory

mediators [75]. The binding of cellular TF to FVII leads to the formation of the TF:FVIIa complex, which when attached to the cell membrane, initiates the activation of the coagulation system [70, 73]. Once formed, the TF:FVIIa complex is a potent activator of FX and FIX, with FXa the preferred substrate for the TF:FVIIa complex, resulting in continuous activation of coagulation [54, 76, 77]. Activation of the TF:FVIIa complex by FXa is the major initiating feedback loop of coagulation, activating circulating FIX into FIXa, resulting in the production of fibrin (blood clot) [78]. With TF essential for haemostasis, excessive concentrations of TF leads to thrombosis formation, thus regulation of TF activity is required to maintain haemostatic balance [73].

2.4.3(2) Tissue Factor Pathway Inhibitor

To regulate and prevent excessive clot formation, coagulation is balanced by several anti-thrombotic mechanisms [79]. TFPI is the major physiological regulator of TF-induced coagulation [64], limiting activation by two rapidly occurring mechanisms. The first is through the inhibition of the TF:FVIIa complex, and the second is by the direct inhibition of FXa (**Figure 2.3**), limiting the proteolytic activity and the amount of thrombin generated [54, 64, 80-82]. TFPI exists as two isoforms; $TFPI_{\alpha}$ and $TFPI_{\beta}$. $TFPI_{\alpha}$ contains a negatively charged N-terminus, three Kunitz-type inhibitory domains (K1, K2, and K3) and a positively charged C-terminus, and is secreted by endothelial cells predominantly within the microvascular endothelium, whereas $TFPI_{\beta}$ comprises of two domains only (K1 and K2) [83, 84]. K1 and K2 bind to FVIIa and FXa, respectively, to inhibit TF-mediated coagulation, whilst K3 binds to Protein S (a cofactor for the direct inhibition of FXa, and in the absence of FXa, a direct inhibitor of TF:FVIIa complex). In addition, $TFPI_{\beta}$ has an altered C-terminus, enabling the binding of $TFPI_{\beta}$ to the endothelium cell surface via a glycosylphosphatidylinositol

anchor [85]. As previously described by Broze [64], TFPI is kinetically “slow”, meaning that inhibition of coagulation activation is not always immediate or tight-binding (referring to the fact that TFPI produces significant inhibition at concentrations near that of the enzyme being inhibited).

2.4.3(3) Thrombin Generation

Following vessel wall injury, blood loss is controlled by the activation of the coagulation cascade. During haemostasis, thrombin (a multifunctional serine protease) is generated rapidly at the site of injury, playing a pivotal role in the physiological formation and removal of thrombi [86, 87]. Thrombin has been described to play an important role within haemostasis and various non-haemostatic processes (see Posma et al. [88] for review), however this literature review will discuss the haemostatic role of thrombin only.

Thrombin is considered the central enzyme and key regulator in coagulation, and is produced by the proteolytic cleavage of prothrombin (the inactive precursor of thrombin) by FXa [89]. Upon vascular injury, thrombin initiates haemostatic activation through the activation of blood platelets (the first responders and therefore regarded as primary haemostasis), resulting in the release of platelet activators (i.e. adenosine diphosphate, serotonin and thromboxane A_2). Secondary haemostasis immediately follows, initiating the coagulation cascade and resulting in the formation of thrombin. With multiple functions reported [88], a key function of thrombin is the conversion of fibrinogen into fibrin monomers, resulting in the formation of a fibrin-rich blood clot, preventing excessive bleeding from occurring [88]. Yet thrombin has been shown to possess a relatively short-half life and is constantly subjected to inhibition [90]. Therefore, a thrombin feedback loop enables the continuous generation of thrombin.

The thrombin feedback loop results in the activation of FXI into FXIa and additionally activates FIX leading to further amplification of the intrinsic cascade [57]. Thrombin then activates cofactors V, VIII (the regulatory cofactor for FIXa), and the FXIII zymogen, enhancing thrombin and fibrin formation. FVIII requires the feedback action of thrombin to enable the formation of the FIXa:FVIIIa complex, which continues to convert FX into activated FX (FXa). For FX to become "activated", the activation of FVIII is required. Once activated, FVIII binds to von Willebrand factor, resulting in further amplification of the coagulation cascade via the formation of thrombin and fibrin [57, 91]. FXa is continuously subjected to rapid inhibition, and is relatively inefficient by itself [92]. Therefore, when activated and combined with FV, the activity of FXa is enhanced, enabling continuous activation of prothrombin, and therefore thrombin, highlighting the importance of the prothrombinase complex [91].

2.4.3(4) Thrombin Anti-thrombin Complex

Anti-thrombin is a serine protease inhibitor and the primary inhibitor of many of the activated coagulation enzymes [27, 55, 70]. Indeed, FIXa, FXa, TF:FVIIa complex and thrombin (key components of the coagulation cascade) are rapidly bound and neutralised by anti-thrombin [70] (**Figure 2.3**). When unbound, anti-thrombin inhibits thrombin and FXa more efficiently then when thrombin and FXa are bound to active complexes. By itself, anti-thrombin is a poor inhibitor of coagulation, with reactions proceeding at extremely slow rates [55, 70]. To limit the formation of thrombin and fibrin in a rapid manner, anti-thrombin must bind with thrombin, resulting in the formation of the thrombin anti-thrombin complex (TAT) [53]. TAT complex levels are also used as a marker of hypercoagulability with high levels of TAT associated with increased thrombin generation [10, 19, 52, 70, 93]**.**

2.4.4 Fibrinolysis

The fibrinolytic system activates simultaneously with blood coagulation to preserve haemostatic balance [94, 95]. It is a tightly controlled series of interactions between various activators and inhibitors, resulting in the degradation and dissolution of fibrinogen and fibrin monomers into fibrin degradation products (FDP) and D-Dimer (the breakdown of cross-linked fibrin, resulting from the action of FXIIIa to form covalent bonds between D Domains of adjacent fibrin monomers) [42, 70, 96].

To ensure adequate activation of the fibrinolytic system, plasminogen, a circulating zymogen, is converted to plasmin (the enzyme responsible for the lysis of fibrin-to-fibrin degradation products, including D-Dimer) and is absorbed into the developing clot by binding to fibrinogen [70, 97]. The primary activator for the conversion of plasminogen to plasmin *in vivo* is tissue plasminogen activator (t-PA), a serine protease produced by the endothelial cells and released upon damage to the vessel wall [57, 70, 98, 99] (**Figure 2.4**). To prevent excessive unregulated plasmin to plasminogen activity, fibrinolytic inhibitors are required. Plasminogen activator inhibitor (PAI-1) (primarily synthesised in endothelial cells), is the primary inhibitor of fibrinolysis and has been shown to rapidly inhibit t-PA [70]. In contrast, when a blood clot is required, a reduction in plasmin generation occurs, favouring fibrin stabilisation and thrombosis formation [41].

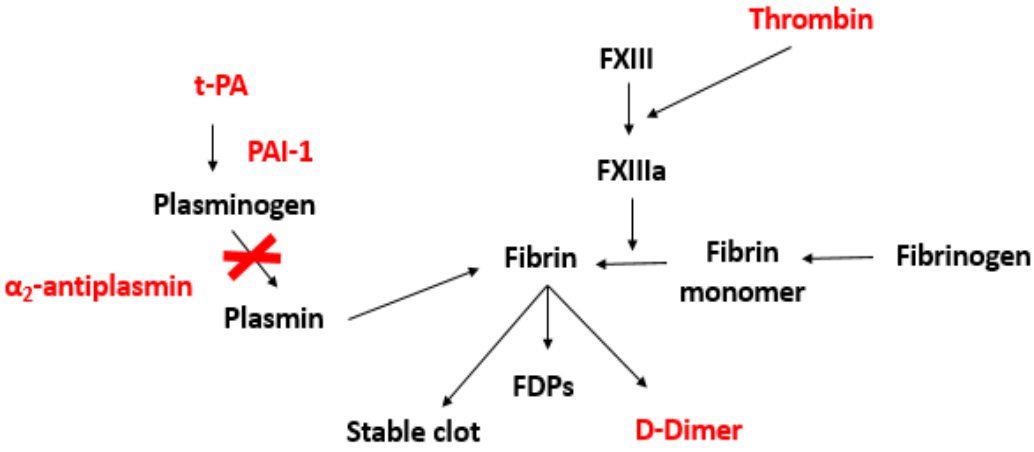


Figure 2.4 *Schematic representation of the fibrinolytic system. Fibrinolysis is initiated by t-PA released into circulation upon endothelial damage. PAI-1 binds to t-PA, inhibiting fibrinolysis. t-PA activates plasminogen to form plasmin in the presence of fibrin; however, this may also be inactivated through binding with* α_2*-antiplasmin. Plasmin degrades the fibrin clot forming FDPs and D-Dimer.* ***Abbreviations:*** *t-PA tissue plasminogen activator; PAI-1 plasminogen activator inhibitor; FDPs fibrin degradation products.*

2.4.4(1) D-Dimer

D-Dimer is a unique marker of endogenous fibrinolysis activation [10, 21, 100, 101] and is clinically used as a marker of fibrin clot formation and fibrinolysis in the exclusion of VTE [102, 103]. D-Dimer, named due to its structure (two cross-linked D fragments of fibrin protein), is a fibrin degradation product formed by the sequential action of three enzymes: thrombin, FXIIIa, and plasmin [1, 104, 105].

Initially and in the presence of thrombin, fibrinogen is proteolytically cleaved into fibrin monomers, promoting the polymerisation of fibrin monomers to one another in an overlapping and cross-linked fashion, serving as a template for FXIIIa and plasmin formation [104, 106, 107]. Additionally, thrombin remains associated with fibrin, assisting with the activation of FXIII to FXIIIa, occurring at the site of the fibrin polymers [105]. The second step of D-Dimer formation, FXIIIa covalently cross-links

fibrin monomers via intermolecular isopeptide bonds [108], whilst in the final step of formation, plasmin cleaves substrate fibrin at specific sites [109].

D-Dimer is currently the best available laboratory marker of coagulation activation, with concentrations of D-Dimer >500ng/mL associated with increased risk of VTE [110, 111] and is therefore frequently investigated in exercise-based studies [15, 102, 112].

2.5. Virchow's Triad and the Athlete

Due to the competition and training demands of competitive sport, it has been postulated that athletes are at an ~85% greater risk for the development and onset of VTE [113], when compared to the general population. This is inconsistent with other reports that suggest overall VTE risk remains relatively low and does not differ between athletes and the general population [32, 114]. Whilst exercise alone is not an overwhelming risk factor for VTE formation, athlete-specific thrombotic risk factors directly relating to Virchow's Triad (hypercoagulability, vessel wall injury and venous stasis (see **Figure 2.5**)), provides indirect evidence as to how VTE may develop within an athletic population. These risk factors are discussed in further detail in sections *2.5.1, 2.5.2* and *2.5.3.* When these athlete-specific thrombotic risk factors are combined with genetic predispositions to blood clotting and oral contraceptive use, athletes may be at an even greater risk for VTE.

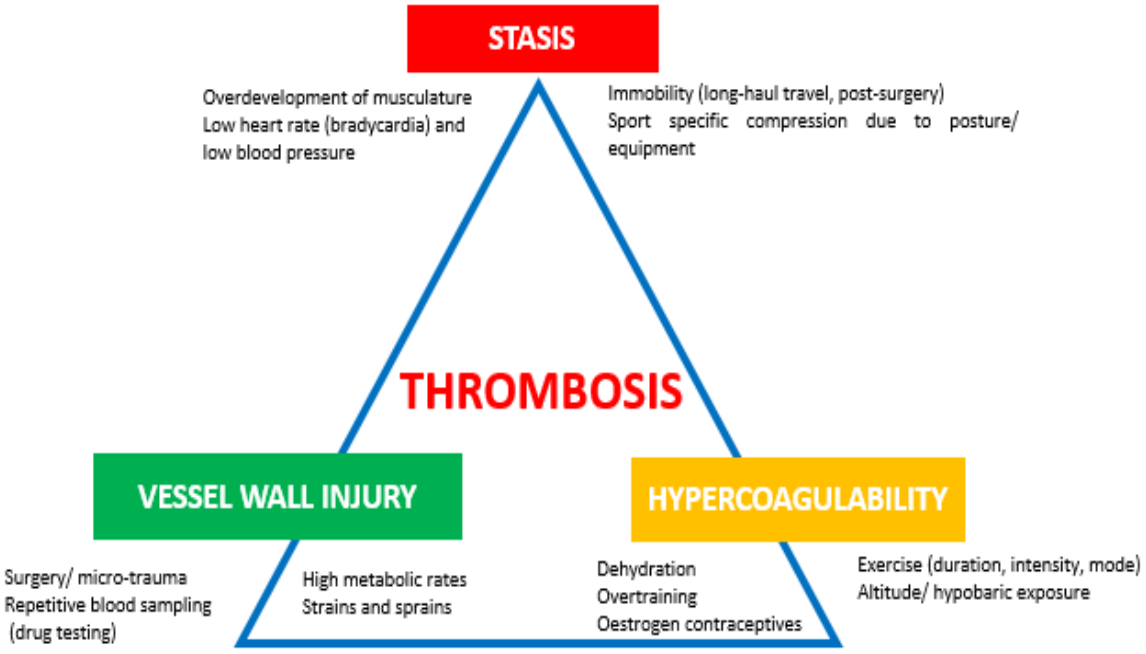


Figure 2.5 *Illustration of athlete-specific thrombotic risk factors in relation to Virchow's Triad, pre-disposing athletes to thrombosis development.*

2.5.1 Hypercoagulability

Hypercoagulability, the increased ability for the blood to coagulate (i.e. clot) has been well documented to occur in athletes due to numerous factors including exercise, dehydration and haemoconcentration, altitude and hypoxic exposure, in addition to oral contrecpetive use in female athletes and performance enhancing supplements, as discussed below.

2.5.1.1 Exercise

Blood is hypercoagulable immediately post-exercise as demonstrated by an increase in markers of *in-vivo* thrombin generation [48, 50, 93]. Hypercoagulability is suggested to occur as a result of increased venous blood flow and increased blood viscosity [115], resulting in increased laminar shear stress directly applied to the vascular walls, releasing TF. This results in the activation of the coagulation cascade

via the TF pathway. The degree of hypercoagulability has been demonstrated to be dependent upon the duration and the intensity of the exercise itself (see *section 2.7* for further information) [10, 19, 93, 112, 116].

2.5.1.2 Dehydration and Haemoconcentration

Dehydration results from insufficient fluid intake in combination with an increase in sweating rate, commonly experienced throughout exercise [5, 117]. Dehydration has been shown to have direct implications on blood hypercoagulability due to haemoconcentration (a thickening of the blood due to an increase in red blood cell concentration and a decrease in plasma volume) [118]. Adequate hydration during- and immediately post-exercise may reduce the potential for haemoconcentration, with the majority of sports providing targeted guidelines to athletes to prevent dehydration. The influence of haemoconcentration on thrombotic episodes remains speculative [119, 120] and may not play a significant role unless combined with other thrombotic risk factors [3].

2.5.1.3 Altitude/Hypoxic Exposure

Increased exposure to moderate-high-altitude as well as hypoxic environments is often required of elite athletes. To improve overall performance outcomes, athletes undertake training at high altitudes within hypoxic environments (~21 days as a consensus for duration) to favourably augment physiological variables. When training at high altitudes and within hypoxic environments, transient increases in haemoglobin concentration (~1% per week) and haematocrit have been demonstrated, increasing the potential for blood hypercoagulability due to a rapid decrease in plasma volume, as well as increases in erythropoiesis and blood viscosity [121]. Indeed, when elite endurance middle-distance runners completed two-weeks of training at a moderate altitude (2000m above sea level), haemoglobin concentrations increased by 2% [122],

with the duration of time spent at high altitude and hypoxic environments directly influencing the concentration of haemoglobin [121, 122]. Training at moderate altitudes (1829 to 3048 m) is frequently adopted by athletes from various sporting disciplines, whilst training at higher altitudes (>3048 m) may be of less benefit due to an increased potential for high altitude sickness [123]. Upon completion of high altitude and hypoxic exposure, erythropoietin is immediately increased, followed by a decrease approximately one-week later [124]. Therefore, an increased potential for hypercoagulability has been suggested to occur within this one-week period post altitude and hypoxic exposure.

2.5.1.4 Oral Contraceptives

Oral contraceptives are widely used by women (and in particular, female athletes) for numerous reasons including birth control and the medical management of amenorrhea and osteoporosis [125]. An abundance of evidence exists showing the use of oral contraceptives activate both coagulation and fibrinolysis as demonstrated by changes in the molecular markers of these systems, resulting at an increased potential for VTE formation [126-129]. Whilst the incidence of VTE occurring in young females (<45 years) using oral contraceptives is relatively low (~ 1 in 3 per 10,000 per year) [130], the combination of oral contraceptives and strenuous exercise have been demonstrated to increase the risk of hypercoagulability [125]. Therefore, female athletes may be at an increased risk for thrombosis formation [125]. When the aforementioned factors are combined with the addition of genetic predispositions to blood clotting, the risk for VTE formation is further increased from 30- to 100-fold as demonstrated in females heterozygous and homozygous for Factor V Leiden mutation.

2.5.1.5 Performance Enhancing Supplements

Throughout the history of sport, a minority of athletes have engaged in unethical practices to obtain an 'edge' over their competitors. Certain practices have been shown to increase the risk of VTE through subsequent decreases in concentrations of anticoagulant and antifibrinolytic markers [131-133]. Anabolic androgenic steroids have been linked to hyperaggregable platelets in addition to a marked increase in cholesterolemia [134], whilst androgens have been demonstrated to augment thrombosis in animal based studies [135]. The use of performance enhancing supplements currently on the prohibited list by the World Anti-Doping Agency [136] include ephedra [133] and erythropoietin [132], which have been shown to increase hypercoagulability, potentially through an increase in blood viscosity.

2.5.2 Vessel Wall Injury

Within an athletic population, endothelial dysfunction/trauma has emerged as the most important factor of Virchow's Triad, promoting thrombosis formation due to its ability to influence the systems present within haemostasis (**Figure 2.2**) [8, 10, 11, 57]. Trauma to the vessel wall may be dependent upon numerous factors including effort induced venous thrombosis (repetitive micro-trauma) and lower limb injuries, and surgery (as discussed in further detail below).

2.5.2.1 Effort-Induced Venous Thrombosis (Repetitive Micro-Trauma)

Numerous studies have reported on effort-induced thrombosis [137-139], referred to as Paget-Schroetter's syndrome. Paget-Schroetter's syndrome is characteristically observed in young athletes (aged 15-30 years, regardless of gender), and is limited to the upper extremity, potentially resulting from strenuous and repetitive exercise involving the affected arm. This results in compression (venous stasis) and micro-trauma (vessel wall injury) directly to the axillary-subclavian vessel wall, either

throughout the thoracic outlet or costo-clavicular space (the first rib) [137, 139]. Repeated trauma to a vein increases inflammation and depositions of fibrin, leading to the progressive narrowing of the vessel, resulting in the activation of the coagulation system [59, 140]. This type of effort-induced VTE is typically seen in sports requiring extensive use of the upper body, and has been reported in rowers, weightlifters, baseball players, gymnasts, basketball players and swimmers [32].

2.5.2.2 Lower-Limb Injuries/ Trauma

DVT within the lower-limb veins (i.e. popliteal, posterior tibial and peroneal veins), typically results from a traumatic event or injury obtained during training and competition [32, 141]. However, the risk of trauma is sport dependent. Non-contact sports result in inadvertent and unexpected trauma, whereas sports with limited contact (baseball, volleyball) pose a higher risk of trauma, with this contact remaining unintentional and occurring infrequently. In contact sports (basketball, soccer) and collision sports (ice hockey, rugby), some degree of trauma routinely occurs during play, providing a greater risk for trauma related injuries. Whether these factors are associated with an increased risk of thrombosis in an athletic population are yet to be determined [142]. In a population based study [143], recent trauma of the lower-limb (including muscle strains and sprains) was demonstrated to be associated with a 13-fold increased risk for VTE. Direct trauma to the lower limb may stimulate the TF pathway of coagulation activation through the release of TF from the vessel wall following injury. The incidence of VTE is at its highest, in the first month following lower-limb injuries [141]. This may result from prolonged periods of immobility due to direct or indirect trauma, further increasing venous stasis and pre-disposing the athlete to thrombin generation.

2.5.2.3 Surgery

With athletes frequently exposed to traumatic injuries (as described above), surgical interventions are routine in nature and may vary throughout the training and competition cycles. Surgery to the lower extremity possesses higher risks for the development and onset of DVT [60], resulting from factors including: 1) the location, type and duration of the surgical intervention performed, 2) direct trauma to the vessel wall, and 3) the recovery timeframe, resulting in prolonged periods of immobility. However, minimally invasive procedures including arthroscopic knee surgery (a common procedure in athletes), are considered to be low to moderate risk for VTE [144].

2.5.3 Venous Stasis

Venous stasis is associated with the slowing of blood flow within the vascular system, and is not considered a risk factor for VTE within an athletic population [59, 142]. Nevertheless, several factors associated with high-level sport may result in venous stasis, promoting venous formation within this population including bradycardia and low blood pressure, overdevelopment of musculature, sports specific compression and prolonged periods of immobility due to long haul travel. These are discussed in further detail below.

2.5.3.1 Bradycardia and Low Blood Pressure

In elite and trained athletes, the cardiovascular system is well developed, undertaking physiological remodelling due to frequent and prolonged bouts of training and exercise [145]. As a result, numerous adaptations occur including left ventricular hypertrophy and a decrease in both heart rate (bradycardia) and blood pressure [36, 145]. This has been postulated to increase VTE risk due to an increased potential for

blood pooling and venous stasis [36]. At present, the relationship between bradycardia, low blood pressure and VTE remains unclear.

2.5.3.2 Overdevelopment of Musculature

Muscular hypertrophy is required within an athletic population to improve overall performance outcomes. Consequently, this may result in compression of the venous system, promoting venous stasis and enhancing VTE risk [3]. Indeed, hypertrophy of the gastrocnemius muscle, may result in entrapment of the popliteal vein (Popliteal Venous Entrapment: PVE), increasing venous stasis and acting as a pathophysiological risk factor for DVT. PVE is especially prevalent in cyclists, with consistent intermittent compression and decompression of the popliteal vein with each revolution of the pedal [146]. Additionally, several case reports of athletes developing arm thrombosis resulting from overdevelopment and hypertrophy of the pectoralis minor and anterior scalene muscles, have been reported (*see section 2.5.2.1 Effort-Induced Venous Thrombosis (Repetitive Micro-Trauma*)) [147, 148], due to the compression of the axilosubclavian artery.

2.5.3.3 Sports Specific Compression

Sports specific compression of blood vessels (resulting in reduced blood flow and venous stasis) may occur as a direct result of postural and equipment requirements. For example, the aerodynamic posture assumed in prolonged periods of cycling has been linked with compression of the external iliac artery [149, 150], whilst Paget-Schroetter Syndrome is extremely common in sports requiring extensive use of the upper limbs [137, 139].

2.5.3.4 Long-Haul Travel and Immobilisation

Athletes frequently undertake long-haul travel for training and competition, resulting in exposure to factors influencing Virchow's Triad. During recent years, the relationship between prolonged travel and VTE has been widely investigated [151, 152], with the incidence of VTE dependent upon the duration and distance of travel [153]. The activation of coagulation has been reported to occur in athletes flying four or more hours [152]. Indeed, prolonged periods of immobility associated with long-haul travel, is thought to result in venous stasis, decreased venous return and haemoconcentration due to the cramped seating environment [154]. Additionally, flight specific factors including the hypobaric and hypoxic nature of air travel may result in activation of the coagulation system [155]. When combined with hypercoagulability (dehydration) and trauma to the vessel wall (possibly through sporting injuries), an increased risk for VTE may be present when undertaking long-haul travel.

2.6 Hereditary and Genetic Mutations

In addition to acquired and athlete-specific risk factors associated with Virchow's Triad (**Figure 2.5**), athletes may possess an inherited or genetic pre-disposition to hypercoagulability, increasing their risk for VTE [3, 60]. When investigating hereditary thrombophilia in 173 elite athletes, 12 cases of activated Protein C resistance (11 heterozygous and 1 homozygous) and 10 cases of Prothrombin G20210A mutation (all heterozygous) were documented [44], potentially placing these athletes a greater risk of VTE when undertaking exercise. [32]. Indeed, De Caterina et al. [156] reported four cases of DVT after strenuous exercise in athletes diagnosed with PC deficiency and APC resistance (aged 24 (x3) and 40 years, respectively).

The two most prevalent forms of genetic thrombophilia include Factor V Leiden and Prothrombin G20210A mutations, resulting in hyperactivity (hypercoagulability) of coagulation factors [32]. Other thrombophilia's including Anti-thrombin III, Protein C and Protein S deficiencies are also associated with an increased thrombotic risk (**Table 2.2**) [157]. Carriers of Protein S, Anti-thrombin III and Protein C deficiencies possess higher risks for the onset of VTE when compared to Factor V Leiden and Prothrombin G20210A (**Table 2.2**). Whilst genetic thrombotic disorders are rare, caution is required for athletes possessing these conditions.

Table 2.2 *Hereditary and genetic forms of thrombophilia: incidence and occurrence of thrombosis formation.*

Hereditary/ Genetic Mutation	What is it?	Probability of developing thrombosis	Occurrence
Protein C deficiency	• A defect in the anticoagulant system, ↑ the risk for thrombosis. • Protein C works with protein S to inactivate the activated forms FV and FVIIIc.	• 8 to 10-fold ↑ risk for carriers [158]	• 0.2-0.4% in the general population [159]
Protein S deficiency	• ↓ levels of Protein S, ↓ its function. • Protein S functions as a cofactor to Protein C in the inactivation of FVa and FVIIIa.	• 8.5 times ↑ risk for carriers [160]	• 0.005-2.3% in the general population [160]
Anti-thrombin III deficiency	• Anti-thrombin III provides anticoagulation effects by inactivating proteins of the coagulation pathway including FVIIa, FIXa, FXa, FXIa, FXIIa and thrombin. • Leads to a higher risk of VTE than PC or PS deficiencies [161]	• 8.1 times ↑ risk for carriers [162]	• 0.02-0.17%; 1 in 2000 individuals [163]
Factor V Leiden Mutation (FVL)	• FVL mutation is the most prevalent inherited thrombophilia [60]. • ~4-6% of the general population are heterozygous.	• 2.2 times ↑ risk for carriers [47]	• 7-fold ↑ risk in heterozygotes [164] • 25-80-fold ↑ risk in homozygotes [47]
Prothrombin G20210A	• Second most common form of inherited thrombophilia. • Prothrombin G202010A ↑ levels of prothrombin by ~one-third.	• 2.8 times ↑ risk for carriers [162]	• 2 to 5-fold ↑ risk in the general population [46]

2.7 Exercise and Haemostasis

Exercise has been recognised to possess a variety of health benefits, including cardiovascular protective elements [165] whilst reducing the development and onset of chronic diseases [39]. Yet exercise also has considerable effects upon haemostasis, resulting in transient increases in blood coagulation [8, 10, 50], platelet aggregation [12] and fibrinolytic activity [8, 13, 14, 40]. Whilst exercise-induced disturbances in the haemostatic balance may not be detrimental to most participants, approximately 1 in 1000 athletes [33, 44] will experience an exercise related thrombotic event, with athletes exposed to many underlying and acquired risk factors associated with the components of Virchow's Triad (**Figure 2.5** and **Table 2.1**).

Haemostasis remains active at low levels to maintain the overall dynamic equilibrium of the haemostatic system. However if the activation of the coagulation and fibrinolytic systems are not balanced, a predisposition to thrombus formation may result, especially when combined with other thrombogenic risk factors [42]. The degree to which the coagulation, platelet and fibrinolytic systems are activated appear to be related to the intensity and duration of the exercise, the study population assessed (i.e. healthy, trained or sedentary, age and gender) [9, 44, 166] and the time of day the exercise is performed [7, 31, 45].

2.7.1 Duration and Intensity

During exercise, the coagulation and fibrinolytic systems activate simultaneously to preserve haemostatic balance [152]. The degree to which these systems are activated is dependent upon the duration and intensity of the exercise [15, 166]. Indeed, activation of the coagulation system as indicated by markers of *in-vivo* thrombin generation, have been shown to increase following extremely short-duration maximal

exercise (90 s) [19] (**Table 2.3**) and longer-duration strenuous exercise (>2 h) (**Table 2.4**) [7, 10, 52]. However, conflicting responses for coagulation activation in regards to exercise duration have been reported [51, 52], suggesting that exercise-induced coagulation activation is most likely dependent on exercise intensity [40]. It is important to note however, that the activation of the coagulation system following exercise may not directly result in the formation of DTV.

2.7.2 Short-Duration, High-Intensity Exercise and Haemostatic Responses

Activation of coagulation due to an increase in post-exercise hypercoagulability, has been well established to occur within short-duration (<60 min) exercise [8, 19, 43, 167] (**Table 2.3**). Yet many of the findings from previous research are difficult to compare due to discrepancies in defining exercise duration, intensity, exercise mode, and the physical training status of the participants. Indeed, when completing extremely short-duration high-intensity exercise (i.e. 15 to 90 s) and exercise of 20 min and longer, conflicting responses of coagulation activation have been reported [8, 18, 19, 51]. In healthy male subjects, coagulation activation was observed following a 15 and 90 s maximal isokinetic exercise test) [18] and a 30 s Wingate test [8]; however, this was not the case following 45 s of maximal isokinetic cycling ($p>0.05$) within the same population [18] (**Table 2.3**). Whilst coagulation activation has been reported in extremely short-duration (90 s) [18] and longer duration exercise (20 min) [19], there is a lack of evidence with regards to coagulation activation in exercise durations between 90 s to 20 min.

The intensity of exercise has been shown to play an influential role in shifting the equilibrium between coagulation and fibrinolysis, with strenuous (i.e. high-intensity) exercise consistently shown to increase haemostatic activation as opposed to low-moderate intensity exercise [17, 40, 49, 50, 112] (**Table 2.3**). When investigating the

influence of exercise intensity on fibrinolytic activity, El-Sayed [168] found 15 min of cycling at 70%VO_{2max} enhanced overall fibrinolytic activity to a greater degree than cycling at 40%VO_{2max}, whilst Szymanski and Pate [17] observed ~50% increase in t-PA activity when cycling 30 min at 80%VO_{2max} when compared to cycling at 50%VO_{2max}. Of interest, little change in overall fibrinolytic activity has been reported by Davies et al. [169] when exercising at intensities below 50-60%VO_{2max}. Similar responses for the activation of coagulation in relation to exercise intensity have been previously reported [11, 49]. Indeed, Weiss et al. [11] found 60 min of running at 83%VO_{2max} resulted in significant activation of the coagulation system when compared to running at 68%VO_{2max}, whilst Menzel and Hilberg [49] observed increased concentrations of TAT when undertaking 60 min of cycling at 100% individual anaerobic threshold (IAT), but not at 80%IAT (**Table 2.3**). Elevated concentrations of catecholamines and higher shear stress associated with increasing exercise intensity may be responsible for the activation of coagulation [170, 171]. Ikarugi et al. [171] found a correlation between elevated coagulation activity with rising norepinephrine concentrations, whereas similar findings were observed by Menzel and Hillberg [49], with significantly greater increases in norepinephrine observed when exercising at ~83%VO_{2max} when compared to ~68%$VO_{2ma.x}$. This suggests that exercise intensity plays an important role in the formation and removal of thrombin through activation of both the coagulation and fibrinolytic systems [172].

When investigating activation of coagulation following graded incremental exercise tests only, conflicting responses for TAT have been reported [19, 51]. Indeed, in exercise of similar intensities and duration, significant increases in post-exercise TAT complexes were observed by Dufaux et al. [19] ($p<0.01$; effects size: 0.33), but not within the study of Hilberg et al. [51] (treadmill: $p>0.05$, effect size: 0.67: cycling:

p>0.05, effect size: 0.81) (**Table 2.3**). Despite the absence of a significant increase in TAT, Hilberg and associates [51] reported significant post-exercise increases in F 1+2 (treadmill: p<0.05; effect size: 0.63; cycling: p<0.05; effect size: 0.92), demonstrating the importance for the correct selection of haemostatic markers to assess coagulation activation.

The selection of coagulation markers play an influential role as the duration of exercise increases. Significant elevations (p<0.05) in TAT have been reported immediately upon completion of a 30 [173] and 60 min run [16, 50] and immediately following 60 min of cycling [49]. Indeed, Weiss et al. [77] demonstrated significant increases in TAT (p=0.001) and F 1+2 (p<0.05) following a 60 min run, however, they failed to demonstrate a significant post-exercise increase in TF (p>0.05, d=0.29). These findings suggest an increase in post-exercise hypercoagulability and potential for VTE following exercise of 60 min or less, with TAT and F 1+2 suitable markers to assess *in-vivo* thrombin generation following exercise.

In conclusion, both the coagulation and fibrinolytic systems are activated simultaneously following short-duration, high-intensity exercise albeit in an apparently healthy, untrained population. Whether haemostatic activation differs within an athletic and well-trained population remains unclear, highlighting the need for future research. To date, there is scarce evidence of coagulation and fibrinolytic responses to an exercise bout between 90 s to 20 min of duration, with this duration of exercise frequently employed in most training and competitive events.

Table 2.3 *Coagulation and fibrinolytic responses to short duration exercise (60 minute and less).*

Author	No. of participants; sex; training status	Blood Sample Collection	Exercise Protocol, Duration and Intensity	Haemostatic Markers Analysed	Main Findings (Effect Sizes)
Hilberg et al. [18]	15; M; healthy	1. Pre-exercise 2. Post-exercise	Isokinetic maximal tests 15 s	• F 1+2 • TAT • t-PA • PAI-1 • D-Dimer	↑* (*d*= 0.20) ↔ (*d*= 0.09) ↑* (*d*= 0.56) ↔ (*d*= -0.06) ↔ (*d*= -0.06)
			45 s	• F 1+2 • TAT • t-PA • PAI-1 • D-Dimer	↔ (*d*= 0.11) ↔ (*d*= -0.34) ↑* (*d*= 0. 77) ↔ (*d*= -0.07) ↔ (*d*= -0.21)
			90 s	• F 1+2 • TAT • t-PA • PAI-1 • D-Dimer	↑* (*d*= 0.31) ↔ (*d*=-0.04) ↑* (*d*= 1.36) ↔ (*d*= -0.01) ↔ (*d*= -0.09)
Gunga et al. [8]	15; M; healthy	1. Pre-exercise 2. post-exercise	30-s anaerobic Wingate test	• PTT • FVIII • t-PA • D-Dimer	↑** (*d*= -1.41) ↑*** (*d*= 1.93) ↑***(*d*= 0.94) ↑*** (*d*= 1.22)
Womack et al. [174]	13; M; healthy	1. Pre-exercise 2. Post-exercise	GXT treadmill test (1 min stages, gradient by 3% per min), mean duration un-reported.	• t-PA • PAI-1	↑* (*d*= 2.23) ↑* (*d*= -1.08)

Author	No. of participants; sex; training status	1. Blood Sample Collection	Exercise Protocol, Duration and Intensity	Haemostatic Markers Analysed	Main Findings (Effect Sizes)
Hilberg et al. [51]	13; M; healthy	2. Pre-exercise 3. Post-exercise	GXT, Treadmill: (3 min stages, 0.5 m/s increments), mean duration: 20.2 ± 2.6 min.	• F 1+2 • TAT	↑* (*d*= 0.63) ↔ (*d*= 0.53)
			GXT, cycling ergometer: (3 min stages, 50W increments), mean duration 19.4 ± 3.4 min.	• F 1+2 • TAT	↑* (*d*= 0.52) ↔ (*d*= 0.48)
Dufaux et al. [19]	11; M; moderately trained	1. 30 min pre-exercise 2. Post-exercise	Incremental GXT (3 min stages, 30W increments) ~24-36 min	• TAT • t-PA	↑** (*d*= 0.85) ↑** (*d*= 3.65)
Hegde et al. [173]	10; M; healthy	1. Pre- exercise 2. Post-exercise	30 min run @70-75%VO_{2max}	• D-Dimer • t-PA • FVIII	↑*** (*d*= 4.00) ↑*** (*d*= 7.74) ↑** (*d*= 2.44)
			30 min walk @ 1.2mph	• D-Dimer • t-PA • FVIII	↔ (*d*= 0.00) ↔ (*d*= -0.16) ↔ (*d*= 0.74)
Molz et al. [14]	10; M, 10; F; healthy	1. Pre-exercise 2. Post-exercise	30 min cycling (10W increments every minute until HR of 120bpm achieved)	• t-PA (M, F) • D-Dimer (M, F)	↑** (*d*= 1.21+0.57) ↑** (*d*= 0.33+0.26)
			Immediately post 30 min cycling, workload increased by 25W every 2 min until HR= 180-190bpm.	• t-PA (M, F) • D-Dimer (M, F)	↑** (*d*= 0.83 +1.15) ↑** (*d*=0.69 +0.62)
Rocker et al. [172]	15; M; healthy	1. Pre-exercise 2. Post-exercise	30 min cycling (10W increments every minute until HR of 130bpm achieved)	• t-PA • F 1+2 • TAT	↑*** (*d*= 0.37) ↑** (*d*= 0.24) ↔ (*d*= 0.10)
			Immediately post 30 min cycling, workload increased by 25W every 2 min until HR= 180-190bpm.	• t-PA • F 1+2 • TAT	↑*** (*d*= 0.73) ↑*** (*d*= 1.20) ↑* (*d*= 0.70)

Author	No. of participants; sex; training status	Blood Sample Collection	Exercise Protocol, Duration and Intensity	Haemostatic Markers Analysed	Main Findings (Effect Sizes)
Herren et al. [48]	18; M; healthy	1. Pre-exercise 2. Within 3 min post-exercise	60 min run (intensity self-selected) (n=10)	• FPA • TAT • F 1+2 • β thromboglobulin	↔(d= 0.72) ↑** (d= 2.62) ↑* (d= 1.08) ↑* (d= 2.55)
			60 min cycling (intensity self-selected) (n=8)	• FPA • TAT • F 1+2 • β thromboglobulin	↔ (d= 1.33) ↑** (d= 2.00) ↑* (d= 0.32) ↑** (d= 1.62)
Hilberg et al. [16]	16; M; healthy	1. 30 min pre-exercise 2. Post-exercise	60 min run (treadmill) at 90% IAT	• F 1+2 • TAT • D-Dimer	↑* (d= 1.10) ↑* (d= 2.02) ↑* (d= 0.80)
Menzel et al. [49]	25; M; healthy	1. Pre-exercise 2. Post-exercise	60 min cycling at 80%IAT	• FVIII • TAT • t-PA Ag • D-Dimer	↑* (d= 0.92) ↔ (d= 0.33) ↑* (d= 1.96) ↑* (d= 0.76)
			60 min cycling at 100%IAT	• FVIII • TAT • t-PA • D-Dimer	↑* (d= 2.08) ↑* (d= 1.08) ↑* (d= 2.97) ↔ (d= -0.21)
Weiss et al. [11]	12; M; well trained	1. After 30 min of exercise 2. Post-exercise	60 min run (treadmill) at 68%VO_{2max}	• t-PA • F 1+2 • TAT • FPA	↑* (d= 9.48) ↔ (d= 0.08) ↔ (d= 0.33) ↔ (d=-0.41)
			60 min run (treadmill) at 83%VO_{2max}	• t-PA • F 1+2 • TAT • FPA	↑* (d=0.33) ↑* (d= 0.26) ↑* (d= 2.80) ↑* (d= 6.34)

Author	No. of participants; sex; training status	Blood Sample Collection	Exercise Protocol, Duration and Intensity	Haemostatic Markers Analysed	Main Findings (Effect Sizes)
Weiss et al. [77]	12; M; endurance trained	1. Pre-exercise 1. Post-exercise	60 min maximal run (treadmill) at velocity corresponding to AnT	• TF • TAT • F 1+2	↔ (*d*= 0.29) ↑** (*d*= 7.33) ↑* (*d*= 4.00)
Weiss et al. [50]	11; M; endurance trained	2. + 30 min pre-exercise 3. ~1 min post-exercise	60 min swimming	• F 1+2 • TAT • FPA	↑* (*d*= 0.29) ↑* (*d*= 3.67) ↑* (*d*= 2.40)
			60 min cycling @75%VO_{2max}	• F 1+2 • TAT • FPA •	↔ (*d*= 0.07) ↔ (*d*= 0.43) ↔ (*d*=0 .55)
			60 min running @ ~77%VO_{2max}	• F 1+2 • TAT • FPA	↑* (*d*= 0.50) ↑* (*d*= 7.00) ↑* (*d*= 4.22)

Abbreviations: * p<0.05; ** p<0.001; ***p<0.0001; ↑ increase; ↓ decrease; ↔ no change; @ at; AnT anaerobic threshold; β beta; bpm beats per minute; *d*=effect size; F females; min minutes; F 1+2 prothrombin fragment 1+2; FPA fibrinopeptide A; FVIII factor 8; h hours; GXT graded exercise test; IAT individual anaerobic threshold; km kilometres; M males; min minutes; m/s meters per second; n number; PC platelet count; PAI-1 plasminogen activator inhibitor; PTT partial thromboplastin time; s seconds; TAT thrombin anti-thrombin complex; TF tissue factor; t-PA tissue plasminogen activator; VO_{2max} maximal oxygen uptake; W watts.

2.7.3 Long-Duration, Low-Moderate Intensity Exercise and Haemostatic Responses

Long-duration low-moderate intensity exercise is continuously growing in popularity, with the completion of events including marathon running, triathlons and ultra-marathons. Indeed, long-duration exercise has been shown to stimulate alterations in the haemostatic system, promoting a hypercoagulable and hyperfibrinolytic state, as evidenced by increased concentrations of TAT, F 1+2, D-Dimer and t-PA [10, 24, 52, 95, 102, 167, 175] (**Table 2.4**).

The co-activation of fibrinolysis is suggested to occur to maintain the dynamic equilibrium of the haemostatic system. This has been shown to be initiated through the release of t-PA from the vascular endothelium [176], due to numerous factors including thrombin [177], vascular shear stress associated with an increase in blood flow [178] and the hormonal release of catecholamines and vasopressin [179]. When fibrinolysis is activated, a significant increase in the concentration of D-Dimer is frequently demonstrated to occur, especially upon immediate completion of long-duration strenuous exercise [21, 102, 175, 176, 180] (**Table 2.4**).

When undertaking a 42.2km marathon run, significant increases in TAT ($p<0.04$), F 1+2 ($p<0.001$), D-Dimer ($p<0.02$) and t-PA ($p<0.01$) were observed by Prisco et al. [10] and Parker et al. [102, 152], despite an overall difference in mean finish time (2.45 ± 0.15 h v 3.38 ± 0.26 h= 0.53 ± 0.11 h, respectively) (**Table 2.4**). Whilst the majority of studies investigating haemostatic responses to marathon running have been conducted on relatively flat terrains, one study by Sumann and associates [95] investigated the influence of a downhill speed marathon on haemostasis, using 13 healthy participants. The findings of Sumann and associates [95] were similar to those of the flat terrain marathon runs, with 2.8- and 1.24-fold increase in hypercoagulability as demonstrated by plasma concentrations of TAT and D-Dimer, respectively, despite

a high percentage of eccentric muscle work. Whilst eccentric muscle exercise is considered less metabolically demanding, eccentric exercise has been shown to induce greater damage in the form of micro-tears to the associated skeletal muscles, altering the extracellular matrix, resulting in an inflammatory response [21, 181]. In addition to the haemostatic responses, Sumann and associates [95] reported a 2.5-fold increase in creatine kinase (CK) (a marker of muscle damage [182]), potentially initiating an inflammatory response. Since there is a strong correlation between the activation of coagulation and inflammation [95, 180], the findings of Sumann and associates [95] are expected, with a high contribution of eccentric work providing a potent stimulus for activation of the haemostatic system. This coagulation activation resulting from the inflammatory response has been proposed to occur via a direct interaction with the TF coagulation pathway [43, 183]. Whilst haemostatic activation is similar regardless of the terrain, the topography of the marathon itself may influence the intensity, load and type of muscle contraction in which the marathon is completed, ultimately determining the degree to which the coagulation and fibrinolytic systems are activated. Conversely, no comparable haemostatic data between a flat terrain marathon and a downhill marathon is available, making comparisons with other long distance runs difficult to interpret, highlighting the need for further research within this area.

As the duration of exercise extends beyond the standard marathon distance (i.e. 42.4km), greater coagulatory, fibrinolytic and muscle damage has been reported. For example, after a 67km mountain run, Schobersberger et al. [21] reported significant increases in markers of TAT (1.4-fold) ($p<0.01$), D-Dimer (1.4-fold) ($p<0.05$), t-PA (3.7-fold) ($p<0.05$) and CK (16.5-fold) ($p<0.001$) in healthy male runners. Similar to the findings of Schobersberger et al. [21], albeit after a run of significantly greater distance (total: 161km), Kupchak et al. [20] also reported significant increases in the

plasma concentrations of TAT (2.4-fold increase; $p<0.001$), F 1+2 (3.9-fold increase; $p<0.001$), D-Dimer (2.2-fold increase; $p<0.001$), t-PA (4-fold increase; $p<0.001$) and CK (154-fold increase; $p<0.05$) from baseline measures. Although these are separate studies, as the distance and the duration is increased from 67km to 161km, the relative increase in the activation of the haemostatic system was larger as demonstrated by greater increases in TAT, D-Dimer and t-PA. In addition, when exercising for a longer duration, extensive muscle damage was reported as shown by 154-fold increase in CK concentrations ($p<0.0001$), potentially initiating the activation of the coagulation system. This therefore suggests that the duration of the exercise bout plays an influential role in the dynamic equilibrium between coagulation and fibrinolysis.

Whilst post-exercise hypercoagulability and hyperfibrinolytic activity are well documented (**Table 2.4**), there are conflicting reports on the duration of haemostatic activation from ~24 h onwards post-exercise. Elevated concentrations of TAT, F 1+2 and D-Dimer have been described the day after completion of long-duration and strenuous exercise bouts [10, 20, 21, 24, 102], suggesting an increased potential for thrombin generation and adverse outcomes within this 24 h period [184]. However, this was not observed in all studies, with Rocker et al. [52], Bartsch et al. [94], Parker et al. [152] and Sumann et al. [95] reporting markers of coagulation to have returned to baseline values within 3, 21, 24 and 24 h respectively, of exercise completion (marathon, triathlon and downhill marathon run) (**Table 2.4**). Within 48 h of exercise completion, markers of haemostasis were typically reported to have returned to baseline values [10, 167] in all but one study [20]. 48 h upon completing an ultra-marathon run, D-Dimer was reported to have remained increased by ~2.1-fold in comparison to baseline measurements, suggesting the time frame of haemostatic activation may be dependent upon the duration (time) and distance (kilometres) of the

exercise bout itself. Indeed, the 161km ultra-marathon in the study of Kupchak et al. [20] was completed in 24.64 hours, providing a possible explanation as to why D-Dimer remained substantially elevated 48 h later.

Table 2.4 *Pre- to Post-exercise haemostatic responses to long-duration (>90 minutes) low-moderate intensity exercise.*

Author	No. of participants; sex; sport	Exercise Protocol, Duration and Intensity	Blood Sample Collection	Haemostatic Markers Analysed	Main Findings
Arai et al. [167]	15; M; triathletes	Triathlon (3.8km swimming, 179.2km cycling, 42.2km run). **Mean finish time**: unreported	1. 48 h pre-exercise 2. Post-exercise	• PT • Fibrinogen • Anti-thrombin III • t-PA • D-Dimer	↔ (1.0-fold) ↑* (1.1-fold) ↔ ↑* (2.9-fold) ↑* (1.9-fold)
Bartsch et al. [94]	10; M; triathletes	Triathlon **Mean duration/ finish time**: 128-163 min	1. 24 h pre-exercise 2. Post-exercise	• TAT • F 1+2 • FPA	↑** (2.1-fold) ↑** (1.5-fold) ↑* (1.8-fold)
Kupchak et al. [20]	12; M, 4; F; ultra-marathon runners	Ultra-marathon (161km trail foot-race) **Mean finish time**: 24.64 h (range: 16.89-29.46 h)	1. 20 h pre-exercise 2. Post-exercise	• F 1+2 • TAT • t-PA • D-Dimer PAI-1	↑* (3.9-fold) ↑* (2.4-fold) ↑* (4.0-fold) ↑* (2.2-fold) ↑* (4.5-fold)
Parker et al. [102]	24; M, 17; F; marathon runners	Boston Marathon run (42.2km) **Mean finish time**: 3.38 ± 0.26 h	1. 24 h pre-exercise 2. Post-exercise	Travel • D-Dimer • P-selectin • Microparticles Control • D-Dimer • P-selectin • Microparticles	 ↑** (2.7-fold) ↑** (1.8-fold) ↑* (2.5-fold) ↑** (2.7-fold) ↑** (1.8-fold) ↑* (2-fold)
Parker et al. [152]	24; M, 17; F; marathon runners	Boston Marathon run (42.2km) **Mean finish time**: 3.38 ± 0.26 h	1 24 h pre-exercise 2 Post-exercise	Travel • TAT • t-PA Control • TAT • t-PA	 ↑** (3.1-fold) ↑** (4.6-fold) ↑** (1.5-fold) ↑** (4.9-fold)
Prisco et al. [10]	12; M; marathon runners	Marathon run (42.2km) **Mean finish time**: 2.45 ± 0.15 h	1. 24 h pre-exercise 2. Post-exercise	• F 1+2 • TAT • t-PA antigen • D-Dimer	↑*** (8.8-fold) ↑*** (12.5-fold) ↑*** (3.5-fold) ↑** (1.9-fold)

Author	No. of participants; sex; sport	Exercise Protocol, Duration and Intensity	Blood Sample Collection	Haemostatic Markers Analysed	Main Findings
Rocker et al. [175]	16; M; marathon runners	Marathon (42.2km) **Mean finish time (range)**: 2.25 to 2.49	1. 30 min pre-exercise 2. Post-exercise	• t-PA • t-PA antigen • PAI-1 • D-Dimer TAT	↑*** (31.0-fold) ↑*** (6.0-fold) ↔ ↑* (1.7-fold) ↑*** (1.8-fold)
Schobersberger et al. [21]	11; M; ultramarathon runners	Ultramarathon (67km)	1. 24 h pre-exercise 2. Post-exercise 3. 24 h post-exercise	• TAT • D-Dimer • t-PA • PAI-1	↑* (1.4-fold) ↑** (1.4-fold) ↑** (3.7-fold) ↔
Sumann et al. [95]	12; M, 1; F; marathon runners	Downhill marathon (795 m vertical difference) **Median finish time**: 223 min (range: 211-247 min)	1. 3 h pre-exercise 2. ~ 30 min post-exercise	• TAT • F 1+2 • D-Dimer • t-PA	↑* (2.8-fold) ↑* (1.35-fold) ↑* (1.24-fold) ↑* (11.0-fold)

Abbreviations: * $p<0.05$; ** $p<0.001$; *** $p>0.0001$; ↑ increase from baseline values; ↓ decrease from post exercise values; ↔ no significant difference ($p>0.05$); F females; F 1+2 prothrombin fragment 1+2; FPA fibrinopeptide A; h hour; km kilometre; M male; m metre; min minute; PAI-1 plasminogen activator inhibitor; TAT thrombin anti-thrombin complexes; t-PA tissue plasminogen activator.

2.8 Circadian Rhythms and Haemostasis

The superchiasmatic nuclei (SCN) within the anterior hypothalamus has been well documented to regulate and maintain circadian rhythms (i.e. the overt expression of an internal timing mechanism measuring daily time) [31] present within numerous biological functions, including core body temperature, the sleep-wake cycle, blood pressure and the synthesis and secretion of several hormones (i.e. melatonin and cortisol) [31, 185, 186]. These intrinsic circadian rhythms are slightly longer than a standard 24 hour day (~24.2 hours), hence the term circadian (*circa* meaning 'around', and *dies* meaning 'day') [186], with circadian rhythms demonstrating peaks and troughs for numerous biological functions. Remarkably, the haemostatic system has been well demonstrated to possess its own circadian rhythms [187]. When measured under resting conditions, fluctuations in coagulation, fibrinolysis, platelet aggregation and blood viscosity are well documented, with increases in hypercoagulability, platelet aggregation, blood viscosity and hypofibrinolysis reported to possess an acrophase (the time at which the peak of a rhythm occurs) between 0600 and 1200 h [7, 25, 26, 31, 45, 188-190] (**Table 2.5**). Therefore, it is unsurprising that numerous cardiovascular diseases [191, 192], cerebrovascular events [193] and pulmonary embolisms [194] possess their own circadian variations, with greater occurrences reported during the morning, and a secondary smaller peak observed in the afternoon [195]. However, the underlying mechanisms of circadian rhythms within haemostasis are not yet fully understood.

Whilst several haemostatic factors appear to be influenced by a variable degree of circadian or diurnal (occurring during the daytime) variations, it remains unclear if every marker associated with the haemostatic system possess their own circadian rhythms [196]. It is suggested that variations in the coagulation and fibrinolytic factors

may influence overall dynamic haemostatic balance [31]. When investigating TAT complex, previous studies have demonstrated inconsistent findings as to whether a circadian rhythm is present [136, 190, 197, 198]. When investigated over several different time points (i.e. 0730 to 2100 h) in healthy male and female individuals, Jafri et al. [190] and Deguchi et al. [136] observed a notable absence of circadian rhythms within TAT ($p>0.05$). Of interest, increased concentrations of TAT were observed at 0900 (ES when compared to 1500h: $d= 0.48$) [136] and 1000 h (ES when compared to 1500h: $d= 0.70$) [190], however these findings were not statistically significant. This may be a direct result of the studies being underpowered with relatively small sample sizes (n=9 [190] and n=10 [136, 190]) reported. It is important to note that in the study of Jafri et al. [190], the peak in TAT at 1000 h was due to abnormally elevated levels in a single participant, with no statistical analysis run with the removal of this individual.

In contrast to TAT, anti-thrombin III (a primary inhibitor of thrombin), has been clearly demonstrated by Casale et al. [197] and Pasqualetti et al. [198] to possess a diurnal rhythm, possessing an early afternoon acrophase (1200 noon) and late evening troughs (the lowest point of a rhythm) when observed in healthy male participants of varying ages (23-87 years). When directly comparing the afternoon acrophase for anti-thrombin III within young (23 ± 3.5 years) and elderly (83 ± 4 years) male participants, a significant increase ($p=0.01$) of ~17.5% in the amplitude of the acrophase was observed within the young males [197] (**Table 2.5**). This variation between the two different age groups is unsurprising, with increasing age generally associated with a reduced circadian amplitude and easily altered circadian acrophase [199]. Therefore, the findings of the abovementioned research confirm the existence of a diurnal rhythm within anti-thrombin III, supporting the notion for a morning increase in

hypercoagulability. With age demonstrated to play a key role in circadian responses for anti-thrombin III [197], future investigations are required to investigate this potential relationship within other markers of haemostasis.

In addition to anti-thrombin III, a clear morning acrophase (0800 h) and afternoon trough (1400 h) has been identified for TFPI and FVII activity, with an 11% and 6% increase respectively, observed within the morning in healthy male subjects [31]. When investigating TFPI, a similar pattern for a morning acrophase in TFPI as reported by Pinotti et al. [31] was observed by Dahm and colleagues [196] (**Table 2.5**). However, no statistical analysis was completed in the study by Dahm and associates [196], therefore these findings should be regarded with caution. In support of a morning peak in FVII are the findings of Kapiotis et al. [45], in which higher values of FVII were observed at 0800 h (2.03 ng/mL, (CI 1.16 to 2.88 ng/mL)), with FVII concentrations significantly decreasing throughout the day to 1.16 ng/mL (CI 0.81 to 1.5 ng/mL; p=.005) at 2000 h. A similar pattern for a morning peak (~0900 h) and an afternoon trough for FVIII and FIX have been identified in three separate studies [26, 45, 200], supporting the notion for a hypercoagulable state within the morning.

In contrast to coagulation, previous studies have shown that overall fibrinolytic activity possess clear diurnal variations, with significantly reduced activity within the morning, whilst peaking in the afternoon and evening [200-202] (**Table 2.5**). This is believed to be due to the diurnal variations present within individual markers of the fibrinolytic system itself, including PAI-1 (the primary inhibitor and regulator of t-PA) and t-PA (the protein responsible for initiating fibrinolysis). Indeed, PAI-1 has been shown to possess significant circadian variations, with two- to four-fold higher concentrations of circulating PAI-1 observed within the morning (0600-0800 h),

versus the afternoon and evening (1800 h) as demonstrated by Juhan-Vague et al. [203], Grimaudo et al. [204], Kluft et al. [202] and Huber et al. [205]. In contrast to PAI-1, t-PA has been well acknowledged to possess opposite troughs and peaks to that of PAI-1. Several studies have demonstrated identical troughs and peaks within healthy adults, with t-PA concentrations (and therefore fibrinolytic activity) at its lowest within the morning, whilst possessing an afternoon acrophase, as demonstrated by higher concentrations of t-PA [200, 202] (**Table 2.5**). With diurnal rhythms well established in PAI-1 and t-PA, data on the diurnal variations within D-Dimer is currently limited, and when reported, often conflicting. When investigated over the course of a 24 h day in healthy adults, Johansen et al. [206] and Jafri et al. [190] failed to demonstrate variations in D-Dimer, whereas, peaks at 0900 and 2300 h ($p<0.05$) in D-Dimer was observed by Iversen et al. [26]. Of interest and the only point of difference between the studies, are the sample collection procedures implemented by the authors, with Johansen et al. [206] and Jafri et al. [190] using repeated venepunctures, whilst indwelling venous cannulas were used within the study by Iversen et al. [26]. When directly comparing the methods of venepuncture and an indwelling cannula on the response to coagulation activation, no significant differences were reported in patients receiving heparin infusion [207], suggesting the method of collection was unlikely to influence the morning increase in D-Dimer concentrations observed by Iversen et al. [26]. Although large variations have been observed within PAI-1 and t-PA, whether a true diurnal variation is present within D-Dimer warrants further investigation, especially due to the clinical significance of D-Dimer.

2.8.1 Haemostasis, Exercise and Time of Day

Although diurnal rhythms have been identified in numerous markers of coagulation and fibrinolysis under resting conditions, the influence of exercise completed at various times of the day and its role on haemostasis, is rarely reported. To investigate the diurnal rhythms in response to exercise completed at different times of the day, three separate investigations [7, 9, 17] have employed similar exercise protocols with varying exercise intensities over two different time points (**Table 2.6**). The findings of Syzmanski and Pate [17] followed the diurnal rhythms previously reported for t-PA and PAI-1, with both markers significantly increased post-exercise ($p<0.05$), with greater concentrations observed immediately following evening exercise ($p<0.001$). In contrast to Szymanski and Pate [208], Siahkouhian and associates [9] failed to demonstrate a time of day response in markers of coagulation and fibrinolysis following both morning and evening exercise, despite significant increases ($p<0.05$) in post-exercise plasma concentrations of haemostasis. When investigating the platelet system in response to exercise at two different times of the day, platelet count, mean platelet count and platelet aggregation were demonstrated to display significant time of day differences, with significantly greater increases observed following morning exercise [7] (**Table 2.6**). Whilst the research into haemostatic responses to exercise at different times of the day is scarce, it is important to note these variations regarding exercise and diurnal rhythms in markers of haemostasis have primarily been investigated within non-athletic, apparently healthy populations, and may not be applicable to a well-trained or athletic population. Indeed, when comparing trained versus untrained individuals, higher fibrinolytic activity has been reported to occur within the trained population [208, 209], whilst no direct comparisons between these populations exist for the platelet and coagulations systems, with investigations limited

to training interventions in untrained individuals only [15, 210, 211]. The time of day at which an athlete exercises is often unavoidable, as they are required to compete and train over the course of a 24 h day based upon the scheduling of training and competition. Therefore, further investigations into coagulation and fibrinolytic responses to exercise completed at varying times of the day within this population are essential and may assist in providing targeted guidelines to this specific population.

Table 2.5 *Diurnal variations in markers of haemostasis (investigated in the absence of exercise).*

Author	No. of participants; sex; age (y)	Time of day (blood sample collection)	Haemostatic markers analysed	Main findings
Angleton et al. [200]	33; M; 31 (21-92 y)	0800 h ± 1 h 1930 h ± 1 h	• t-PA activity • PAI-1	Evening peak, morning trough ($p<0.01$) Morning peak, evening trough ($p<0.01$)
Casale et al. [197]	Young: 6; M; 23 (± 3.5y) Aged: 6; M; 83 (± 4y)	0000 h 0400 h 0800 h 1200 h 1600 h 2000 h	Young • Anti-thrombin III Aged • Anti-thrombin III	Afternoon peak (1200 h) ($p=0.008$)*** Afternoon peak (1200 h) ($p=0.01$)
Dahm et al. [196]	8; M; undisclosed	1 hourly (0700-1200 h) 2 hourly (1200- 1800 h)	• TFPI	Mid-morning to afternoon peak (1000 to 1600 h)
Deguchi et al. [136]	10; M; undisclosed	0900 h 1200 h 1500 h	• TAT	↔ greater TAT concentrations at 0900 vs 1200 & 1500 h
Grimaudo et al. [204]	8; M; undisclosed	0800 h 1000 h 1200 h 1600 h 2000 h	• t-PA • PAI-1	Evening peak, morning trough ($p<0.01$) Morning peak, evening trough ($p<0.01$)
Huber et al. [205]	11; M; 20-38 y	0600 h 1200 h 1800 h	• PAI-1 • t-PA antigen	Morning peak, evening trough ($p<0.05$) Morning peak. evening trough ($p<0.05$)
Iversen et al. [26]	8; M; undisclosed	12 separate times over 24 h (times not specified)	• F 1+2 • FVII • D-Dimer • PAI-1 • vWF	Bimodal peak at 0900 h and 2300 h ↔ Bimodal peak at 0900 h and 2300 h Morning peak, evening trough ↔
Jafri et al. [190]	4; M, 5; F; 51 ± 10 (51 ± 10 y)	D1: 1500, 1800, 2100 h D2: 0700, 0800 h	• TAT • D-Dimer	↔ ↔

Author	No. of participants; sex; age (y)	Time of day (blood sample collection)	Haemostatic markers analysed	Main findings
Juhan-Vague et al. [203]	10; M; 10; F; 34 (24-55 y)	0800 h 1600 h	• PAI-1 antigen • t-PA antigen	morning peak, afternoon trough ($p<0.01$) morning peak, afternoon trough ($p<0.001$)
Kapiotis et al. [45]	5; M, 5; F; 19-40 y	0800 h 1200 h 1600 h 2000 h	• FVIIa • F 1+2 • PAI-1	morning peak, evening trough ($p=0.005$) morning peak, evening trough ($p=0.005$) morning peak, evening trough ($p<0.005$)
Kluft et al. [202]	10; M; 22-34 y	0900 h 1200 h 1500 h	• t-PA activity • t-PA antigen • PAI-1 antigen • PAI-1 activity	afternoon peak, morning trough ($p<0.05$) morning peak, evening trough ($p<0.05$) morning peak, evening trough ($p<0.05$) morning peak, evening trough ($p<0.05$)
Pasqualetti et al. [198]	15; undisclosed	3 hourly over a 24 h (midnight →)	• Anti-thrombin III	mid-day/ early afternoon peak ($p<0.001$)
Pinotti et al. [31]	13; M; 25-35 y	0800 h 1400 h	• TFPI • FVII	morning peak, afternoon trough ($p<0.05$) morning peak, afternoon trough ($p<0.05$)

Abbreviations: ******* Significant difference ($p=0.01$) in amplitude observed between young and aged; ↔ no circadian/ diurnal rhythm present; D day; F females; F factor; F 1+2 prothrombin fragment 1+2; h hour; M males; n number; PAI -1 plasminogen activator inhibitor; TAT thrombin anti-thrombin complexes; TFPI tissue factor pathway inhibitor; t-PA tissue plasminogen activator; FVII factor seven; FVIIa activated factor seven; FVIII:C factor eight antigen; vs versus; vWF von Willebrand factor; y years.

Table 2.6 *Haemostatic responses to exercise at different times of the day.*

Author	No. of participants; sex; fitness level	Study design		Blood sample collection	Haemostatic markers analysed	Main findings (pre- to post-exercise)
		Exercise protocol	Time of day			
Aldemir et al. [7]	10; M; moderately/ active	30 min submax cycling (70%VO_{2max})	0730 h 1700 h	• Pre-exercise • Post-exercise • 30 min post-exercise	• PC • MPV • PA	↑m ↓e ↓m
Siahkouhian et al. [9]	15; M; healthy/ sedentary	30 min submax cycling (70%VO_{2max})	0730-0830 h 1730-1830 h	• Pre-exercise • Post-exercise • 30 min post-exercise	• PC • aPTT • PT • t-PA • PAI-1	↔ ↔ ↔ ↔ ↔
Szymanski and Pate [208]	14; M	30 min submax exercise (50%VO_{2max})	0630-1000 h 1600-1900 h	• Pre- exercise • Post-exercise	• t-PA • PAI-1	↑e ↑e

Abbreviations: ↔ no time of day effect; ↑e significantly higher ($p<0.05$) in evening trials; ↑m significantly higher ($p<0.05$) in morning trials; ↓e significant decrease ($p<0.05$) post-evening trials; ↓m significant decrease ($p<0.05$) post-morning trials; aPTT activated partial thromboplastin time; h hour; M males; min minute; MPV mean platelet count; PA platelet aggregation; PAI-1 plasminogen activator inhibitor 1; PC platelet count; PT partial thromboplastin time; submax submaximal; t-PA tissue plasminogen activator; VO_{2max} maximal oxygen uptake.

2.9 Compression and Haemostasis

2.9.1 Overview

Compression garments (i.e. stockings, pneumatic compression devices, compression bandaging) have been used in clinical based settings as the preferred prophylaxis [212, 213] in the prevention and treatment of DVT [214] pulmonary embolism and lymphedema [215, 216], and in the management of wound, scar and venous leg ulcers [217]. Numerous studies have demonstrated an increase in deep venous velocity, reduced venous stasis, and an improved venous return (components associated with Virchow's Triad) in hospital patients when compression garments were worn [212, 218-221]. Additionally, the use of compression garments have been demonstrated to maintain the dynamic balance between the coagulation and fibrinolytic systems, through increases in both arterial [222] and venous blood flow [223]. Compression garments apply a mechanical pressure at the bodies' surface, directly applied to the muscle, bone and connective tissue, resulting in a reduced transmural pressure of the superficial veins. This then causes the superficial veins to dilate, redistributing superficial blood flow from the peripheries to the deep venous system, resulting in improved venous blood flow and peripheral circulation [219, 224].

The efficacy of compression garments may be dependent on different factors. including; their style (i.e. stocking, sleeves, upper and lower body garments) [225], size, and the method of compression. This includes graduated (highest pressure exerted at the distal end of the limb, decreasing to a lower pressure exerted at the proximal end of the limb) [222], uniform (even pressure applied to both the proximal and distal ends), or progressive (pressure is greatest at the proximal end of the limb, decreasing to a lower pressure at the distal end of the limb) compression [219].

Graduated compression has been shown to be “most beneficial” for reducing venous pooling and improving venous blood flow velocity within a clinical setting [219, 222, 226]. Despite a lack of clear scientific evidence on the effects of compression garments on physiology, their use within the sporting industry continues to grow in popularity due to proposed ergogenic effects for exercise performance and recovery [227, 228].

2.9.2 Compression Garments and Haemostasis

With exercise well documented to disturb the haemostatic balance (*see section 2.7. Exercise and Haemostasis*), research on the use of compression garments to maintain a favourable haemostatic state within, and immediately following exercise is relatively scarce. Indeed, Zaleski and colleagues [24] aimed to investigate the influence of compression socks on haemostatic responses when worn during the 2013 Hartford Marathon (42.2km). Endurance-trained runners were randomly allocated to a compression (compression socks worn during the marathon; n=10) or control group (no compression socks worn during the marathon; n=10) and blood was collected from runners pre- and post-marathon. When worn during the marathon, compression socks failed to significantly lower overall haemostatic activation, yet significantly lower concentrations of t-PA in the sock versus control group (sock: 8.9 ± 0.7 ng/ml, control: 11.2 ± 0.7 ng/ml p=0.04) were observed. Whilst not significantly different (p=0.07), average TAT tended to be lower in runners assigned to the sock group (2.8 ± 0.2 µg/L) when compared to the control group (3.4 ± 0.2 µg/L), with the authors concluding that compression socks do not have a negative impact on haemostatic activation and overall haemostatic balance. Additionally, Zaleski and associates [24] highlighted the need for studies with a larger sample size to confirm the preliminary findings of their small investigation.

Of interest, Zaleski and associates [229] also conducted a case study investigating the effect of compression socks on haemostatic activation in an individual with a predisposed susceptibility to thrombosis formation. Within this case study, a single female endurance athlete heterozygous for Factor V Leiden mutation (7-fold increased risk for thrombosis (**Table 2.2**)) completed two separate marathons, one marathon wearing compression socks (2XU Compression Performance Run Sock; 19-25mmHg at the ankle) and one marathon without compression socks. When compression socks were worn, haemostatic activation was reduced as demonstrated by lower post-exercise concentrations of t-PA (~56%), TAT (~63%) and D-Dimer (~30%), suggesting compression reduced the risk for thrombosis in an athlete with a genetic mutation for hypercoagulability. This case study was not without limitations as the two marathons were completed three years apart (control marathon: 2010 and compression sock marathon: 2013), whilst the course elevation of the two marathons also varied greatly (control marathon: 470 feet, sock marathon: 101 feet). The findings of this case study should be interpreted with caution, and highlights the need for tighter-controlled studies within populations pre-disposed to blood clotting.

Whilst the findings of Zaleski et al. [24, 229] have "set the scene" for research into the effect of compression socks on haemostatic responses in endurance exercise, further research investigating haemostatic responses with a larger sample size representative of the general population is warranted. With long-duration endurance-based exercise continuously growing in popularity and frequently favoured by master level athletes, the potential to prevent adverse thrombotic events upon exercise completion is of the utmost importance. It has been widely reported that markers of coagulation including F 1+2, TAT complex, and D-Dimer are altered with increasing age, with the risk of VTE two to five-times higher in people aged 60 years and over, and four to six–times

greater in individuals aged 70 years and older [230]. Compression garments may mitigate the potential for exercise associated VTE risk and therefore further investigation is required.

3.0 Literature Review: Conclusion

The review of literature in this chapter has critiqued the role of haemostasis and the factors that may influence haemostasis. More specifically, the review focuses on numerous factors that may influence coagulation responses within a well-trained population including the duration and intensity of exercise, the time of day exercise is performed, and coagulation and fibrinolytic responses to the use of compression garments during exercise.

This review has identified four key markers of haemostasis frequently used in the assessment of coagulation and fibrinolysis in exercise-based studies: TAT, TF, TFPI, and D-Dimer. These markers represent key stages in blood coagulation, i.e. initiation (TF) and regulation (TFPI), thrombin generation (TAT) and fibrinolysis (D-Dimer), and will be central to the studies presented in chapters five and six.

Although exercise has numerous health benefits, it has considerable effects on haemostasis, including transient increases in blood coagulation, platelet aggregation and fibrinolytic activity. The degree of activation of these pathways is dependent on the duration and intensity of the exercise bout [51, 52], the study population (i.e. healthy, trained or sedentary, age and gender) [9, 44, 166] and the time of day the exercise is performed [7, 31, 45]. Whilst the activation of coagulation during exercise of very short duration (15-90 s) [8, 18] and longer duration (> 20 min) [20, 21, 95] has been identified within this literature review, a lack of consensus for responses to exercise with durations of 90 s to 20 min currently exists. Of particular interest, the

research surrounding haemostatic responses to exercise is performed using apparently healthy and untrained participants only, and as such, coagulatory responses to exercise within an athletic population are unknown.

The time of day in which exercise is performed may also play a key role in the degree of haemostatic activation. Within the literature, significant increases in coagulation with a corresponding decrease in fibrinolysis in the absence of exercise have been reported, between 0600 and 1200 h [7, 25, 26, 31, 45]. It is therefore likely an increase in coagulation responses should be observed within this period when undertaking exercise; however, only three separate investigations have sought to investigate this theory [7, 9, 17]. Upon completion of a morning and evening exercise bout, the author's findings confirm the presence of a morning peak and afternoon trough in coagulation and platelet aggregation, with a morning trough and afternoon peak observed in fibrinolytic parameters. Yet similar limitations as reported for exercise duration and intensity exist for the examination of time of day influences on haemostasis including similar duration of exercise bouts (all 30 min), and the training status of study participants (untrained or apparently healthy individuals). With current time of day training suggestions provided for "at-risk" individuals (i.e. afternoon exercise with the avoidance of morning exercise [30]), these suggestions are often unrealistic in athletic populations, with athletes required to compete and train over the course of a 24 h day. Whether the degree of haemostatic activation varies over the course of a day within an athletic population remains unknown and of interest.

Due to the proposed benefits of compression garments including improved venous velocity, venous return and venous stasis within a clinical population [212, 218-221], it has been suggested that similar outcomes will be observed with the transfer of compression garments towards a sport setting. This transfer has arisen due to the

potential for improved performance and recovery outcomes and not for the potential haemostatic protective measures. Furthermore, whether compression garments influence the haemostatic balance when combined with exercise is currently scarce. To date, only a single case study [229] and one original investigation [24] has been conducted, investigating the influence of compression garments on haemostasis when combined with endurance-based exercise [24], with the authors reporting positive findings with an overall decrease in haemostatic activation reported. Of interest was the finding for TAT, with no significant differences observed between groups (compression socks worn versus not worn during a marathon run). With TAT considered the gold standard in the haematological assessment of thrombin generation, further studies investigating the use of compression socks in prolonged endurance exercise and its effect on TAT warrants further investigation to examine the protective effects of compression garments against VTE when combined with endurance exercise.

Upon critical review of the literature, it is clear that questions relating to factors that may influence haemostasis within a well-trained and athletic population (including exercise duration and intensity, the time of day exercise is performed and the use of compression garments during prolonged-exercise), remain unanswered. Therefore, the series of studies within this thesis aimed to address the following questions:

1. In well-trained cyclists, does a short-duration high-intensity exercise bout influence the coagulation and fibrinolytic systems and do these responses vary when exercising at different times of the day?

2. Is a diurnal response present within TAT, TF, TFPI and D-Dimer in well-trained athletes? and

3. Can compression socks worn during a marathon (42.2km) alter the activation and balance of the haemostatic system?

Chapter Three: Validity of power settings of the Wahoo KICKR Power Trainer

An original version of this chapter has been published in the International Journal of Sports Physiology and Performance as a brief review and appears in the literature as:

Zadow, E.K., Kitic, C.M., Wu, S.S.X., Smith, S.T. & Fell, J.W. "Validity of power settings of the Wahoo KICKR Power Trainer". *International Journal of Sports Physiology and Performance,* 2016, 11, 1115-1117.

Journal Impact Factor: 2.654

Rationale

In order to enable the investigation of coagulation activation in a short-duration high-intensity bout of exercise (chapter five), a cycling ergometer with the ability to provide valid measures of power (a key measure of performance), was required. Ergometers that replicate cycling are important items of laboratory equipment and when used with cyclists' own bicycles, is critical for ecological validity (the ability to accurately replicate the requirements of cycling), producing reliability of testing protocols, whilst removing a potential haemostasis variable (due to cycling position). This study was the first to assess the validity of power settings of the Wahoo KICKR Power Trainer and was therefore deemed most appropriate in the investigation of haemostatic activation within study three.

3.1 Abstract

Purpose: The purpose of this study was to assess the validity of power output settings of the Wahoo KICKR Power Trainer (KICKR) using a dynamic calibration rig (CALRIG) over a range of power outputs and cadences. ***Methods:*** Using the KICKR to set power outputs, powers of 100-999W were assessed at cadences (controlled by the CALRIG) of 80, 90, 100, 110 and 120rpm. ***Results:*** The KICKR displayed accurate measurements of power between 250-700W at cadences of 80-120rpm with a bias of -1.1% (95%LoA: -3.6-1.4%). A larger mean bias in power was observed across the full range of power tested, 100-999W 4.2% (95%LoA: -20.1-28.6%), due to larger biases between 100-200W and 750-999W (4.5%, 95%LoA: -2.3-11.3% and 13.0%, 95%LoA: -24.4-50.3%), respectively. ***Conclusion:*** When compared to a CALRIG, the Wahoo KICKR Power Trainer has acceptable accuracy reporting a small mean bias and narrow limits of agreement in the measurement of power output between 250-700W at cadences of 80-120rpm. Caution should be applied by coaches and sports scientists when using the KICKR at power outputs of <200W and >750W due to the greater variability in recorded power.

Keywords: cycling, power, ergometry, calibration, training

3.2 Introduction

With changes in cycling performance as small as 1% determining the difference between a finish on the podium as opposed to a finish within the peloton, the ability to accurately monitor training and competitive performances in highly trained cyclists is of high importance [231]. Ergometers that replicate cycling are important pieces of laboratory equipment that can be used to conduct fitness assessments, enable structured training sessions and monitor training responses [232]. With stationary laboratory ergometers, the resistance may be generated through mechanical friction [232], air resistance [233], or electromagnetism [234] in order to replicate the physiological demands of cycling. Standard stationary laboratory ergometers have demonstrated various limitations including the inability to precisely replicate the setup of a cyclist's own bicycle (i.e. same components, dimensions, gearing and joint angles) [233, 235] and the dynamic demands associated with cycling on the road [234]. Given that cyclists may ride in excess of 35,000km per year, the ability to replicate individual training and race-specific variables as closely as possible is highly desirable [233]. Indeed, the use of a cyclist's own bicycle in performance assessment as suggested by Paton and Hopkins [235], is critical for producing reliable results predictive of competitive performance. When accustomed to the exercise protocol and cyclists' own bicycles are used, the ability to replicate the physiological demands of cycling [236] and movement economy are improved, enhancing the ecological validity in measures of performance [234, 236, 237].

To track meaningful changes in competitive performance from an ergogenic or training intervention, Hopkins et al. [237, 238] have suggested ergometer error/bias should be less than 2%. As such, the smaller reported coefficients of variation when a cyclist's own bicycle is used are more likely to meet this stringent requirement within

performance assessments. To manipulate training techniques and to detect meaningful changes in performance, coaches and sports scientists need to be confident in the validity and reliability of power outputs of the cycling ergometers being used [231, 239].

A recently available mobile cycle ergometer is the Wahoo KICKR Power Trainer (KICKR: Wahoo Fitness, Atlanta, GA), a Bluetooth 4.0 and ANT + enabled stationary power trainer allowing for the use of cyclists' own bicycles attached via a SRAM/Shimano 10-speed cassette with electronic resistance provided. The KICKR is widely used amongst professional and recreational cyclists, however, to date, there is no independent scientific investigation examining the validity of power output generated by the KICKR. Therefore, the aim of the present study was to assess the validity of power measurements provided by the KICKR using a dynamic calibration device.

3.3 Methods

The validity of the KICKR power output was assessed by comparison with the power output of a dynamic calibration rig (CALRIG: Flinders University, Dynamic Calibrator 34118, Adelaide, Australia) at the crank of an attached bicycle, allowing a comparison of power data with an ecologically valid reference point based on first principles [240]. As per manufacturer requirements to overcome potential frictional losses, the CALRIG and the KICKR was operated for 30 min at 100-120rpm and 15 min at 80-100rpm, respectively, immediately prior to the assessment of power over varying cadences. The assessment of power was conducted in standard laboratory conditions (18°C and 40% relative humidity).

The CALRIG used within this study has been previously described [236, 239] and is used to facilitate the application of a constant cadence to the crank rather than to set a power output. The CALRIG then quantifies the reaction torque (N.m) produced by the ergometer tested using a calibrated load cell, 1 m from the fulcrum point with angular velocity (Rad.s-1) recorded via a crystal-based timing device (10 MHz oscillator) [234]. A double universal joint connected to the axis of rotation (i.e. bottom bracket axle) via a spline drive-coupling device delivers the rotational mechanical drive. A bicycle was attached to the KICKR via the SRAM/Shimano Cassette. A range of power outputs (100-999W) were achieved by manually varying the resistance settings to the maximum power achievable (999W) within the Wahoo Fitness Application for the KICKR (Wahoo Fitness, 2014 (version 5.1.1)) using the 'ergometer mode' selection. Power output was increased by 50W every 3 min over 80, 90, 100, 110 and 120rpm. Power produced by the CALRIG was sampled at 200Hz and recorded every second (1Hz), however, only data produced in the final minute of each stage was used for analysis purposes according to the methodology as used by Hopker et al. [241]. A standardised twenty-minute cool down was provided between each cadence tested.

3.4 Statistical Analyses

Measurements of validity (KICKR) were determined using bias and 95% limits of agreement (LoA) in accordance with the methods of Bland and Altman [242]. Data was analysed using GraphPad Prism 5 version 5.03 (La Jolla, CA, USA). The relative error in measurement of power of the KICKR was calculated by subtracting the power measured by the CALRIG from the power settings entered into the Wahoo KICKR. For the purpose of this study, relative measurement bias of <1.5%, 1.5-2.5% and >2.5% were deemed as highly accurate, moderately accurate and inaccurate, respectively [234].

3.5 Results

Overall absolute and relative bias and 95%LoA between the KICKR and the CALRIG over 100-999W and 80-120rpm as determined by Bland-Altman analysis are presented in **Table 3.1**.

Absolute mean power (W) differences in measures of power from the KICKR to the CALRIG over 100-999W at 80-120rpm are presented in **Figure 3.1**. An increased difference between set and measured power above 750W was observed for cadences of 80, 90 and 100rpm (**Figure 3.2**).

Average relative error (%) in measures of power from the dynamic calibration rig to the Wahoo KICKR Power Trainer over 80-120rpm are presented in Figure 2. Larger differences between the KICKR and CALRIG were observed at higher ranges of power (900-999W) at 80, 90, 100 and 110rpm (**Figure 3.2**).

3.6 Discussion

The present study is the first to assess the validity of power measurements provided by the Wahoo KICKR Power Trainer using a CALRIG. The results of this investigation show the KICKR has a small mean bias and narrow limits of agreement in the measurement of power output over 250-700W at cadences of 80-120rpm, with larger mean biases and wider limits of agreements observed at lower and higher set power outputs.

The importance of measured power output for detecting changes within performance have been emphasised previously by Hopkins et al. [237, 238] who suggested to detect meaningful changes in performance due to ergogenic or training interventions in elite athletes, ergometer errors of <2% are required [237, 238]. When compared to a CALRIG, the mean bias of the KICKR of -1.1% (95%LoA: -3.5-1.4%, **Table 3.1**)

over 250-700W at cadences of 80-120rpm, falls within this recommended range for ergometer error. Our findings for the KICKR are consistent with the ergometer errors of the Velotron and SRM power meters of <1% in constant power trials of 250W and 414W in comparison with a CALRIG as reported by Abbiss et al. [234]. Furthermore, an error of <1.3% for incremental power tests performed on the Velotron in comparison to a CALRIG was reported between 400W and 700W, similar to the KICKR's accepted range of power output (250-700W). Larger mean biases and wider LoAs of 4.5% (95%LoA: -2.3-11.3%) and 13.0% (95%LoA:-24.4-50.3%) were observed for powers of 100-200W and 750-999W (**Table 3.1**), falling outside of the acceptable recommended ergometer error [237]. The KICKR's mean bias in the power range of 250-700W is better than the reported biases of 2.3% and -2.5% for the SRM and PowerTap between 50-1000W [239]. Similar to Gardner et al. [239], greater variance in power at the lower and higher ends of measured power was observed across cadences of 80-120rpm.

Despite the overall larger mean biases and limits of agreements observed across cadences at the lower (<250W) and higher (>750W) ranges of power (**Table 3.1**), it is clear these values are influenced by cadence selection. When evaluating cadences independently, **Figure 3.2** shows a small mean bias of 0.8% between the KICKR and the CALRIG at the lower ranges of power (150-200W) at a cadence of 80rpm, and a bias of 0.9% can be seen at 120rpm at the higher range of power (750-950W), both falling within the accepted range for ergometer error as recommend by Hopkins [237]. Due to the stochastic nature of cycling and the constantly changing cadences at varying intensities, the validity of power across combined cadences have been reported for the present study's findings.

3.7 Conclusion

The KICKR provides valid readings of power output over 250-700W at cadences of 80-120rpm. The KICKR appears to be suitable for laboratory training, performance assessments and talent identification purposes. Caution should be applied however at the lower and higher ranges of power (<200 and >750W) with the KICKR recording larger absolute and relative errors in comparison to the CALRIG. With power assessed to the highest operational range of the CALRIG, the validity of power output exceeding 999W (common in sprinting) remains to be investigated.

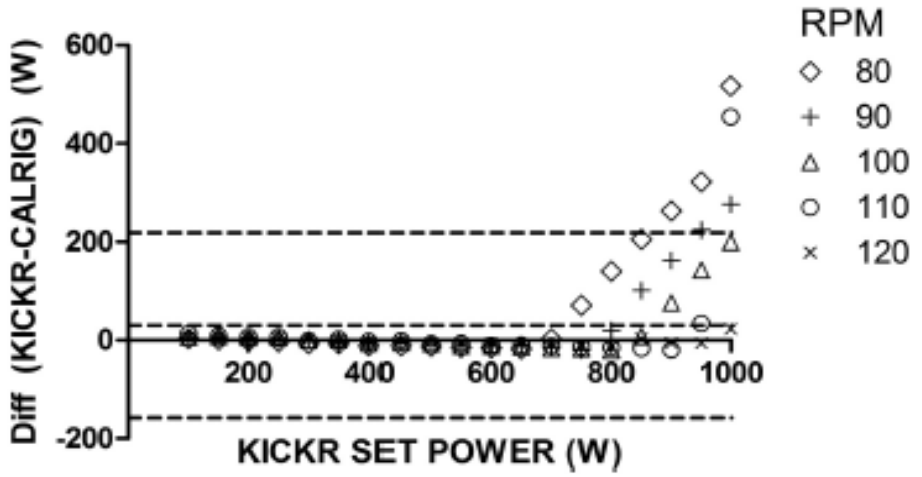
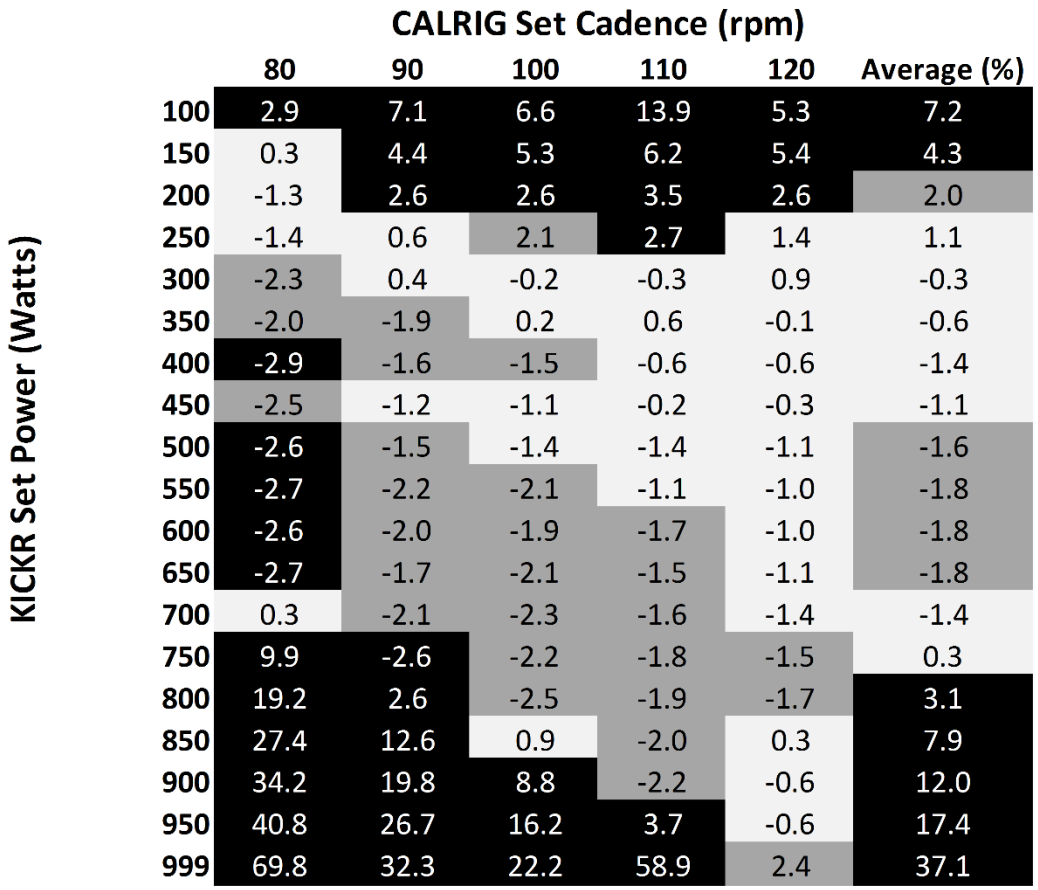


Figure 3. 1 *Bland-Altman plot of the difference in absolute mean power output between the Wahoo KICKR Power Trainer (KICKR) and the dynamic calibration rig (CALRIG) at 80, 90, 100, 110, and 120rpm. Dashed lines represent the mean bias and 95% limits of agreement. Abbreviation: diff, difference.*



	80	90	100	110	120	Average (%)
100	2.9	7.1	6.6	13.9	5.3	7.2
150	0.3	4.4	5.3	6.2	5.4	4.3
200	-1.3	2.6	2.6	3.5	2.6	2.0
250	-1.4	0.6	2.1	2.7	1.4	1.1
300	-2.3	0.4	-0.2	-0.3	0.9	-0.3
350	-2.0	-1.9	0.2	0.6	-0.1	-0.6
400	-2.9	-1.6	-1.5	-0.6	-0.6	-1.4
450	-2.5	-1.2	-1.1	-0.2	-0.3	-1.1
500	-2.6	-1.5	-1.4	-1.4	-1.1	-1.6
550	-2.7	-2.2	-2.1	-1.1	-1.0	-1.8
600	-2.6	-2.0	-1.9	-1.7	-1.0	-1.8
650	-2.7	-1.7	-2.1	-1.5	-1.1	-1.8
700	0.3	-2.1	-2.3	-1.6	-1.4	-1.4
750	9.9	-2.6	-2.2	-1.8	-1.5	0.3
800	19.2	2.6	-2.5	-1.9	-1.7	3.1
850	27.4	12.6	0.9	-2.0	0.3	7.9
900	34.2	19.8	8.8	-2.2	-0.6	12.0
950	40.8	26.7	16.2	3.7	-0.6	17.4
999	69.8	32.3	22.2	58.9	2.4	37.1

Figure 3. 2 *Relative error (%) of the Wahoo KICKR Power Trainer (KICKR) in comparison with the power produced by the dynamic calibration rig (CALRIG) over 100-999W at cadences of 80-120rpm. Errors of <1.5%, 1.5-2.5% and >2.5% are colour coded as light grey, dark grey, and black respectively.*

Table 3. 1 *Bias and 95% LoA for absolute (W) and relative (%) differences in recordings of power at 100-999W for combined cadences of 80-120rpm.*

Power (W)	Bias (95% LoA) (W)	Bias (95% LoA) (%)
100–999	30.0 (–158.0 to 218.1)	4.2 (–20.1 to 28.6)
100–200	5.7 (–1.4 to 12.8)	4.5 (–2.3 to 11.3)
250–700	–6.3 (–18.2 to 5.7)	–1.1 (–3.5 to 1.4)
750–999	102.7 (–186.2 to 391.7)	13.0 (–24.4 to 50.3)

Abbreviation: LoA, limits of agreement.

Chapter Four: The reliability of a laboratory-based 4km cycle time trial on a Wahoo KICKR Power Trainer

An original version of this chapter has been published in the open access Journal of Science and Cycling as an original investigation and appears in the literature as:

Zadow, E.K., Fell, J.W. & Kitic, C.M. "The reliability of a laboratory-based 4km cycle time trial on a Wahoo KICKR Power Trainer". *Journal of Science and Cycling*, 2016, 5, 3, 23-27.

Rationale

Once the KICKR was demonstrated to provide valid measures of power output, the reliability of a short-duration high-intensity bout of exercise (i.e. the 4km cycling time trial) was then required. Time trials (TT) have been shown to provide reliable measures of performance, possessing relatively low measurement errors, with these errors reduced when assessed on repeated occasions. Additionally when cyclists own bicycles are used and attached to a cycling ergometer, the ability to replicate the physiological demands of cycling and movement economy are improved, enhancing the ecological validity of the time trial. Therefore, due to the reliability of the 4km TT when using the KICKR, the 4km TT was deemed suitable for use within the investigation of coagulation responses to TTs when completed on repeated occasions (study three, chapter five). While it would have been practical to publish a single validity and reliability publication, the International Journal of Sports Physiology and Performance requested the two components be split, with the validity of the Wahoo KICKR Power Trainer submitted as a brief report.

4.1 Abstract

The purpose of the present study was to evaluate the reliability of a laboratory-based 4km cycling time trial using a Wahoo KICKR Power Trainer. Twelve trained male cyclists (age: 34.0 ± 6.5 years; height: 1.78 ± 0.62 m; training per week: 11.9 ± 2.6 hours) completed three 4km time trials on the Wahoo KICKR Power Trainer, with each time trial separated by a minimum of two days. During all time trials, mean power (W), cadence (rpm), speed ($km.h^{-1}$), heart rate (bpm) and total time (s) were recorded with rating of perceived exertion (6-20) collected immediately post time trial. Average Intraclass Correlation Coefficients (ICC) between time trials (2v1, 3v2, 3v1) for power was 0.94 (95%CI: 0.85-0.98), cadence 0.73 (95%CI: 0.46-0.90), speed 0.54 (95%CI: 0.22-0.82), heart rate 0.93 (95%CI: 0.84-0.98) and total time 0.64 (95%CI: 0.34-0.86). Mean reliability expressed as the coefficient of variation (CV) and typical error of measurement over the three time trials was 3.4%, 5.2%, 4.2%, 1.6% and 4.3% for power, cadence, speed, heart rate and total time, respectively. Average power measured during a laboratory-based 4km cycling time trial is highly reliable in trained cyclists making it a reliable method for monitoring cycling performance, however, caution should be applied when assessing cadence, speed and total time due to the larger typical errors when completed on the Wahoo KICKR Power Trainer.

Keywords: reproducibility, power output, cycling, athletic performance, performance test

4.2 Introduction

In professional cycling, small differences in performance can often determine the difference between a finish on the podium and a finish within the peloton; therefore, the ability to monitor training and competitive performance changes in highly trained cyclists is of high importance [231, 243]. To detect these meaningful changes within power output, laboratory based performance tests which replicate competitive performances have been shown to possess good test-retest reliability and have the precision to detect changes as small as 1% [237, 243, 244]. In addition, the knowledge of test-retest reliability may determine how sensitive a test is to monitor changes in performance, inform sample size calculations for research studies and enable the comparison of ergometer precision [237].

Time trials (TTs), in which athletes complete a set amount of work in as short a time as possible, have been shown to provide coaches and sports scientists with the ability to monitor responses and detect changes associated with training and fatigue whilst providing accurate representations of the bioenergetics required in competitive cycling [237, 245]. In a review of the validity, reliability and sensitivity of measures of sporting performance, TTs of distances ranging from 5 to 40km, have been shown to be highly reliable in well-trained cyclists with coefficient of variations (CVs) of less than 5% [243], with smaller CVs of 1.9-2.4% observed when completed on cyclists' own bicycles using a Kingcycle™ and SRM™ ergometer [246].

When accustomed to the exercise protocol and cyclists' own bicycles are used, the ability to replicate the physiological demands of cycling [236] and movement economy [247] are improved, enhancing the ecological validity in measures of performance [234, 237, 247]. Indeed, Lamberts et al. [231] reported low CVs of 0.7% and 1.7% in both 40km performance time and 40km mean power in cyclists who rode

their own bicycle on an electromagnetically braked cycle ergometer. The use of a cyclist's own bicycle in performance assessments as suggested by Paton and Hopkins [235], is critical for producing reliable results predictive of competitive performance.

The Wahoo KICKR Power Trainer (KICKR) is an electromagnetically braked portable ergometer, which allows cyclists to use their own bicycles. Indeed, the KICKR has been shown to provide valid measures of power [248], falling within the recommended range of ergometer error of <2% [237, 238]. However, the reliability of a laboratory-based 4km cycle time trial (TT) with cyclists using their own bicycle on the KICKR has yet to be reported. A 4km TT may simulate real world performance such as the 4 000m individual and team pursuit that features at the UCI Track Cycling World Championships. Therefore, the aim of the present study was to determine the reliability of a 4km cycling TT when completed on a Wahoo KICKR Power Trainer in trained cyclists.

4.3 Methods

4.3.1 Participants

Twelve trained male cyclists (age: 34.0 ± 6.5 years, height: 1.78 ± 0.62 m, body mass: 76.8 ± 9.6 kg) with minimum weekly cycling duration of 10 h and previous TT experience volunteered to participate in the study. Participants were provided with written description of the risks and benefits of this study and provided signed informed consent. Ethics was obtained from the Institutional Human Research Ethics Committee. Furthermore, this study conforms to the ethical standards of the Journal of Science and Cycling [249].

4.3.2 Study Design

In a repeated measures study design, participants performed three 4km cycling TTs in standard laboratory conditions (19 ± 1.2ºC and 45 ± 7.5% relative humidity) over three separate occasions within a two-week period. Testing sessions were separated by a minimum of 48 and a maximum of 72 h with testing performed at the same time of day (± 1 h) to minimise the effects of diurnal variation. Participants were required to avoid strenuous activity (<24 h) and caffeine (<12 h) before and on the day of testing. Participants completed a 24 h food diary prior to their first visit and were required to replicate their diet as closely as possible before each subsequent visit. All sessions were performed using the same equipment.

All TTs were performed on participants' own bicycles fitted to the Wahoo KICKR Power Trainer (KICKR: Wahoo Fitness, Atlanta, GA). The KICKR was calibrated using the Wahoo Fitness Utility Application (Wahoo Fitness, 2014, version 2.5) prior to and immediately post each participant's warm up before each TT. For a successful calibration, participants were required to reach a speed of 35.4km.h^{-1} (22 mph) and cease pedalling until 16.0 km.h^{-1} (10 mph) had been reached.

A ten-minute self-selected intensity warm-up immediately followed the calibration process with participants free to alter their pedalling cadence and gear ratio as required. Immediately post warm-up, participants re-calibrated the KICKR with a standardised 60 s period of passive recovery provided following the re-calibration period, with a 10 s non-verbal countdown beginning the TT. Participants were instructed to perform the 4km TTs as fast as possible, commencing from a standing start position with no rolling resistance. Only feedback on distance elapsed was provided throughout each TT. A ten-minute cool-down of self-selected intensity immediately commenced upon completion of the 4km TT. During each TT, heart rate

was recorded at a beat by beat frequency (Wahoo Fitness Blue HR, Atlanta, GA) with speed, cadence and power output recorded at a frequency of 1 Hz via the Wahoo Fitness Application for the KICKR (Wahoo Fitness, 2014, version 5.1.1) and Wahoo Fitness Blue Speed and Cadence (Wahoo Fitness, Atlanta, GA), respectively. Rating of perceived exertion (RPE) for the TT was determined upon immediate completion of the TT using a 6-20 Borg Scale [250].

4.4 Statistical Analyses

Average power, cadence, speed, heart rate and total time between TTs were logarithmically transformed and evaluated using Intraclass Correlation Coefficient (ICC) in combination with 95% confidence intervals (CI), analysed using an Excel spreadsheet for reliability [251]. Thresholds for assigning qualitative terms to the strength of within participant intraclass correlations were as follows: 0.5-0.69 low; 0.7-0.79 moderate; 0.8-0.89 high; 0.9-1.0 nearly perfect [252]. Typical error expressed as a CV% of an absolute value with upper and lower 95% CI were examined between TTs using the Excel spreadsheet of Hopkins [251]. Based on previous research [237], a coefficient of variation lower than 3.5% was regarded as having high test-retest reliability. A one-way within-subject's analysis of variance was used to assess differences in power output between trials using SPSS v23.0, with significance accepted at $p<0.05$. Due to a loss of signal of the cadence sensor as a result of operator error on one occasion, cadence data was obtained from 11 of the 12 cyclists.

4.5 Results

Mean (±SD) data for measures of power, total time, cadence, speed, heart rate and post-exercise ratings of perceived exertion for TTs 1, 2 and 3 are presented in **Table 4.1**. The ICC for mean power and heart rate were nearly perfect at 0.97 (95%CI: 0.92-0.99) with CVs < 2.4% between TT 1 and 2 (**Table 4.2**). Moderate to high ICCs of

0.87 (95%CI: 0.58-0.96), 0.70 (95%CI: 0.23-0.90) and 0.77 (95%CI: 0.36-0.93) were reported for cadence, speed and total time between TT 2 and 3 (**Table 4.2**). The average ICC and CV with 95%CI for measurements between TTs (2v1, 3v2, and 3v1) are presented in **Table 4.2**. Power output during the three time trials was not significantly different (p=0.051).

4.6 Discussion

The present study is the first to determine the reliability of a 4km cycle TT in trained cyclists on the Wahoo KICKR Power Trainer. The results of this investigation show that power within a 4km cycle TT when completed on the KICKR is highly reliable with a CV of 3.4% whereas measures of cadence, speed and total time were shown to be unreliable measures of performance with CV's of 5.2%, 4.2% and 4.3%, respectively.

The importance of detecting performance changes in athletes has been previously emphasized in a review by Hopkins et al. [237] with measures of performance required to show a strong relationship with competitive cycling performance [238, 246, 253]. Laboratory based TTs are frequently used to detect these changes due to the low coefficients of variation (<5%) observed when using various laboratory ergometers [237, 243]. When power output is the key performance variable in repeated TTs, lower CVs of 1.9-3.6% [246, 253, 254] are reported, indicating better test-retest reliability and a more direct method for monitoring of exercise performance [255]. For a 4km TT completed on the KICKR, our findings indicate this cycling test to be highly reliable for power output. We observed a mean CV of 3.4% (CI: 2.4-4.7%) and a within-subject ICC of 0.94 (CI: 0.89-0.98) across three TTs, with the lowest CV (2.4%; CI: 1.7-4.0%) observed between TTs one and two (**Table 4.2**). While highly

reliable, further research is required to quantify how changes in 4km TT on the KICKR reflect changes in on road performance.

In the investigation of both short and longer duration TTs, mean power output for competitive and well-trained cyclists have been observed to consistently exceed 250W, falling within the acceptable range of ergometer error for the Wahoo KICKR Power Trainer (250-700W and 80-120rpm) [248]. Indeed, on average, powers of 346± 38W, 323± 35W and 303± 35W have been reported for a 4, 20 and 40 km cycling TT, respectively [246, 253, 256]. The average power of 344± 41W (**Table 4.1**) observed within the present study is consistent with previously reported power outputs for TTs of varying distances.

To determine the reliability between TTs (within-subject variation), coefficient of variations for power output (2v1, 3v2, and 3v1) was evaluated (**Table 4.2**). Based upon previous studies investigating multiple performance TTs, learning effects have been proposed to contribute to lower CVs observed between TTs two and three (0.9-2.1%) with larger CVs observed between TTs one and two (2.1-3.0%) [253, 254, 257, 258]. To ensure the changes observed within repeated performances are not the result of a learning effect [259, 260], a single familiarisation session has been shown to establish a high degree of reliability and should precede experimental TTs [236, 254]. In contrast to previous findings, there appeared to be no learning effect within the present study in the absence of a familiarisation session with a larger CV of 3.8% (CI: 2.7-6.5%) observed between TTs two to three when compared to TTs one to two (CV: 2.4%, CI: 1.7-4.0%) (Table 2). It has been previously suggested that a familiarisation session may not always be necessary in trained cyclist [235, 253] with the lack of any learning effect from TT one to TT two observed due to the training status and previous TT experience of the cyclists recruited [261]. The larger CV observed between TTs

two to three can be attributable to an increase in average power output observed within the third and final TT (**Table 4.1**). With our findings similar to those observed by Jeukendrup et al. [261] in which performance was greater in the final TT, we propose the improved performance may be due to the participants knowledge that this was their final TT and thus may have been more motivated to complete this TT [258].

With performance time popular in the assessment of reliability within cycling tests due to the ease of measurement, Sporer et al. [253], Palmer et al. [262] and Schabort et al .[263] have demonstrated time to be a reliable method for assessing laboratory based cycling TTs, with CVs of 0.8-1.7% for TTs ranging from 20 to 100 km in trained cyclists. The CV for total time to complete a 4km cycling TT in our study contrasts previous findings with a CV of 4.3% observed. In combination with the large typical errors observed within speed and cadence (**Table 4.2**), this suggests total time, speed and cadence should not be used as key performance outcomes when assessing cycling performance when using the KICKR.

In conclusion, the mean power for a 4km TT performed on the Wahoo KICKR Power Trainer is highly reliable in trained cyclists. With a mean CV of 3.4% for power, a 4km TT on the KICKR is able to detect cycling performance changes of 1.7% in trained cyclists.

4.7 Practical Applications

A 4km TT performed on the Wahoo KICKR Power Trainer is a reliable and therefore sensitive test for coaches and sports scientists to monitor responses associated with performance, training, fatigue and ergogenic aid use within trained cyclists when monitoring and measuring power output only. When assessing 4km TT performance on the Wahoo KICKR Power Trainer, cadence, speed and total time should not be

used due to greater variations in reliability observed within this study. The ability for cyclists to use their own bicycles when attached to the Wahoo KICKR Power Trainer is highly advantageous and critical for producing reliable results predictive of competitive performance.

Table 4.1 *Mean and standard deviation for total time (s), power (W), cadence (rpm) and heart rate (bpm) measured during each 4km cycling time trial (TT). Ratings of perceived exertion (RPE) were measured upon completion of the 4km TT.*

	TT 1	**TT 2**	**TT 3**	**Average**
Total Time (s)	416.4 ± 22.8	421.0 ± 29.9	410.8 ± 27.6	416.1 ± 26.8
Power (W)	342 ± 42	341 ± 45	349 ± 37	344 ± 41
Cadence (rpm)	92 ± 10	93 ± 7	91 ± 8	92 ± 8
Heart Rate (bpm)	174 ± 9	172 ± 8	171 ± 9	172 ± 9
RPE	18.4 ± 1.6	18.1 ± 1.7	18.5 ± 1.5	18.3 ± 1.6

Note that cadence n = 11.

Table 4.2 *Mean within-participant intraclass correlation (ICC) and coefficient of variation (CV) between time trials. Data are presented as mean (95%CI).*

	Power (W)	**Cadence (rpm)**	**Speed ($km.h^{-1}$)**	**Heart Rate (bpm)**	**Total Time (s)**
ICC	0.97	0.78	0.36	0.98	0.51
(2 to 1)	(0.91- 0.99)	(0.36- 0.93)	(-0.24- 0.76)	(0.94- 1.00)	(-0.07- 0.84)
ICC	0.92	0.87	0.70	0.91	0.77
(3 to 2)	(0.75- 0.98)	(0.58- 0.96)	(0.23-0.90)	(0.70- 0.97)	(0.36- 0.93)
ICC	0.80	0.34	0.49	0.84	0.52
(3 to 1)	(0.45- 0.94)	(-0.29- 0.77)	(-0.08- 0.82)	(0.34- 0.29)	(-0.02- 0.84)
Mean	**0.94**	**0.73**	**0.54**	**0.93**	**0.64**
	(0.85- 0.98)	**(0.46- 0.90)**	**(0.22- 0.81)**	**(0.84- 0.98)**	**(0.34- 0.86)**
CV	2.4	4.9	4.5	0.8	5.0
(2 to 1)	(1.7- 4.0)	(3.4- 8.8)	(3.1- 7.7)	(0.6- 1.4)	(3.4- 8.9)
CV	3.8	3.5	3.9	1.8	3.7
(3 to 2)	(2.7- 6.5)	(2.4- 6.2)	(2.7- 6.7)	(1.3- 3.3)	(2.6- 6.7)
CV	3.8	6.8	4.4	1.8	4.1
(3 to 1)	(2.7- 6.5)	(4.7- 12.2)	(3.1- 7.5)	(1.3- 3.2)	(2.9- 7.4)
Mean	**3.4**	**5.2**	**4.2**	**1.6**	**4.3**
	(2.7- 4.7)	**(4.1- 7.4)**	**(3.3- 5.9)**	**(1.2- 2.2)**	**(3.4- 6.1)**

Note that cadence n = 11.

Chapter Five: Time of day and short-duration high-intensity exercise influences on coagulation and fibrinolysis

This manuscript has been accepted as an original research investigation in the European Journal of Sports Science (Accepted December 15, 2017) and will appear in the literature as:

Zadow, E.K., Kitic, C.M., Wu, S.S.X., Fell, J.W. and Adams, M.J. "Time of day and short-duration high-intensity exercise influences on coagulation and fibrinolysis" *European Journal of Sports Science.*

Journal Impact Factor: 2.69

Rationale

With the KICKR and a 4km cycling TT shown to be valid and reliable for research purposes (chapter three and four), both were then used to investigate coagulation and fibrinolytic responses to a short-duration high-intensity bout of exercise in well-trained cyclists. In addition, markers of coagulation and fibrinolysis have been shown to possess circadian rhythms, often peaking within the morning (0600-1200 h). Therefore, the time of day exercise is performed may influence the activation of the coagulation and fibrinolytic systems, with research currently scare within this field, especially when investigated in a well-trained population.

5.1 Abstract

Exercise has been demonstrated to have considerable effects upon haemostasis with activation dependent upon the duration and intensity of the exercise bout. In addition, markers of coagulation and fibrinolysis have been shown to possess circadian rhythms, peaking within the morning (0600-1200 h). Therefore, the time of day exercise is performed may influence the activation of the coagulation and fibrinolytic systems. This study examined the effect of time of day and short-duration high-intensity exercise responses to coagulation and fibrinolysis. Fifteen male cyclists (VO_{2max}: 60.3 ± 8.1 $ml.kg^{-1}.min^{-1}$) completed a 4km cycling time trial (TT) on five separate occasions at 0830, 1130, 1430, 1730 and 2030 h. Venous blood samples were obtained pre- and immediately post-exercise, and analysed for tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombin anti-thrombin complexes (TAT) and D-Dimer. Exercise significantly increases plasma concentrations of TF ($p<0.0005$), TFPI ($p<0.0006$), TAT complexes ($p<0.0012$), and D-Dimer ($p=<0.0003$). There was a time of day effect in pre-exercise TF ($p=0.004$) and TFPI ($p=0.031$), with 0830 greater than 1730 (p+0.001), while 1730 was less than 2030 ($p=0.008$), respectively. There was no significant effect of time of day for TAT ($p=.364$) and D-Dimer ($p=.228$). Power output, TT time and heart rate were not significantly different between TTs ($p>0.05$); however, percentage VO_{2max} was greater at 1730 when compared to 2030 h ($p=0.04$). Due to a time of day effect present within TF, peaking at 0830, caution should be applied when prescribing short-duration high-intensity exercise bouts within the morning in populations predisposed to hypercoagulability.

Key words: Thrombin anti-thrombin III, D-Dimer, circadian rhythm, cycling

5.2 Introduction

Exercise has long been recognised to have considerable effects upon haemostasis, with transient increases in blood coagulation [8, 10, 11], platelet aggregation [12] and fibrinolytic activity [8, 13, 14] well documented and observed to occur in both a healthy and clinical population (i.e. those possessing peripheral arterial disease) [40, 264]. With haemostasis constantly active at low levels, the degree to which the coagulation, platelet and fibrinolytic systems are activated appear to be related to the intensity and duration of the exercise and the study population assessed [15, 51]. Indeed, activation of the coagulation system as indicated by markers of *in-vivo* thrombin generation, i.e. thrombin anti-thrombin complexes (TAT) and prothrombin fragment 1 + 2, have been shown to increase following both short-duration maximal exercise (<1h @~83% of VO_{2max}) [19, 52, 172] and longer-duration strenuous exercise (>2h @ ~90% individual anaerobic threshold) [7, 10, 172]. However, conflicting responses for activation of coagulation, as indicated by TAT complexes, have been observed following short duration exercise of varying intensities [51, 52], with exercise performed at higher intensities resulting in significantly greater concentrations of TAT, suggesting coagulation activation occurs in an intensity dependent manner [40]. Other confounding factors such as the time of day in which the exercise is performed and individual responses to exercise however, may ultimately influence the overall coagulation response.

It is well established that haemostatic markers are subject to circadian rhythms, with hypercoagulability, hypofibrinolysis and increased blood viscosity observed to occur, between 0600 and 1200 h [7, 25-27, 31, 45, 188, 190]. With an increase in the frequency of thromboembolic events reported to coincide with changes in haemostatic variables [28, 29, 265], and with exercise shown to activate the coagulation system [8,

10, 11], it has been suggested that exercise during the afternoon is the safest time of day to exercise, whilst early morning exercise should be avoided completely [30]. This recommendation remains directed at an "at risk/ untrained" population with no current guidelines for well-trained athletes and athletes at more risk of hypercoagulability, including master's athletes and those with genetic mutations for blood clotting.

The time of day at which an athlete exercises is often unavoidable as they are required to compete and train over the course of a 24-hour day based upon the scheduling of training and competition [266, 267]. Although numerous studies have investigated circadian variations in response to exercise [266-269], it remains unclear if coagulation responses to short-duration high-intensity exercise vary at different times of the day within a well-trained athletic population. Therefore, the primary aim of this study was to examine acute changes in the activation of coagulation following short-duration high-intensity exercise at 0830, 1130, 1430, 1730 and 2030 h in well-trained male cyclists. In addition, we aimed to investigate if a time of day effect (circadian variation) was present within markers of haemostasis (Thrombin Anti-thrombin complex and D-Dimer, tissue factor (TF) and tissue factor pathway inhibitor (TFPI)).

5.3 Methods

5.3.1 Participants

Fifteen well-trained male cyclists (mean ± SD: age: 41.5 ± 10.0 years, height: 1.8 ± 0.6 m, body mass: 79.5 ± 9.4 kg, BMI: 24.2 ± 2.3 kg/m^2, VO_2max: 60.3 ± 8.1 ml.kg^{-1}.min^{-1}) with previous TT experience and cycling a minimum of 10 h per week, volunteered to participate in the study. All participants were non-smokers, with no known history or clinical signs of metabolic conditions or coagulation disorders. Subjects were excluded from the study if they had a previous history of thromboembolism. Enrolled participants refrained from using medications that could

affect coagulation (i.e., aspirin and non-steroidal anti-inflammatory drugs) at least 2 weeks before and throughout the testing period [102]. Participants were provided with a written description of the risks and benefits of the study and provided signed informed consent prior to their inclusion within the study. Any details that may disclose the identity of our participants have been omitted. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

5.3.2 Study Overview

Each participant was required to perform seven exercise sessions, no less than 2 and no greater than 7 days apart. Participants were required to avoid strenuous activity and caffeine (<24 h) before and on the day of testing itself, whilst avoiding food 3 h prior to testing. Participants completed a 24 h food and training diary for the day prior to their first visit and were required to replicate their diet and training as closely as possible before each subsequent visit. All exercise sessions were performed on the same equipment, using participants' own bicycles fitted to the Wahoo KICKR Power Trainer (KICKR: Wahoo Fitness, GA, USA) which has been shown to be valid and reliable [248]. The 4km TT employed for this study has previously been shown to provide reliable results when completed on the Wahoo KICKR Power Trainer [270].

5.3.3 Preliminary Testing and Familiarisation

During the initial exercise session, participants completed an incremental maximal exercise test to determine VO_{2max}. The initial workload was 70W with increases of $30W.min^{-1}$. Expired air was collected throughout the maximal exercise test using a metabolic cart (Parvo TrueOne; Parvomedics, UT, USA) at a frequency of 1 Hz and expressed as 30 s mean values. The metabolic cart was calibrated before each test

using alpha gases of known concentrations, according to the manufacturer's requirements.

Participants were familiarised with the laboratory environment and experimental exercise session (excluding the collection of venous blood) during their second visit to the laboratory.

5.3.4 Experimental Sessions

Experimental sessions three to seven required participants to complete a 4km cycling time trial (TT) over five different times of the day (0830, 1130, 1430, 1730 and 2030 h) in standard laboratory conditions (20.8 ± 1.4°C and 46.6 ± 6.2% relative humidity). The order of these exercise sessions was randomised and counterbalanced. Exercise sessions began with ten minutes of supine rest to collect resting measures of oxygen uptake (Parvo TrueOne, Parvomedics, UT, USA) and heart rate collected at a beat by beat frequency (Polar FT4; Polar; Kempele, Finland). Pre-exercise venous blood samples were collected immediately prior to exercise, along with the measurement of blood pressure (Aneroid Sphygmomanometer; Reister; Jungingen, Germany), lactate (Lactate Pro; Arkray, Kyoto, Japan) and tympanic temperature (Covidien Genius 2; Medtronic; Sheffield, United Kingdom).

The exercise protocol consisted of a standardised ten minute warm up (5 min at 50%, 3 min at 70% and 2 min at 80% of peak power measured during the incremental maximal exercise test), followed by 5 minutes of passive rest. Participants then completed a 4km TT as fast as possible, with heart rate recorded at a beat-by-beat frequency, and expired gases, power output, speed and cadence collected at a frequency of 1 Hz. Rating of perceived exertion (RPE) and effort provided for the TT

were determined upon immediate completion of the TT using a 6-20 Borg Scale [250] and a 0-10 (0= no effort, 10= maximal effort) visual analogue scale, respectively.

Immediately upon TT completion, participants were required to lay in a supine position for a further ten minutes with the second (post-exercise) venous blood sample collected immediately at the beginning of the ten-minute period, along with the collection of blood pressure, lactate and tympanic temperature.

5.3.5 Blood Sample Collection

Blood samples were drawn without stasis by venepuncture from the antecubital fossa from participants in a supine position. Blood samples for determining markers of blood coagulation were collected into 3.8% sodium citrate Vacutainers® (Becton Dickinson, New Jersey, USA). Within 30 minutes of collection, citrated blood samples were centrifuged at room temperature for 15 minutes at 2,000 g. Plasma samples were aliquoted and stored at -80^{o}C until required for analysis.

5.3.6 Blood Sample Analyses

Tissue factor (TF), total tissue factor pathway inhibitor antigen (TFPI), thrombin anti-thrombin complexes (TAT) and D-Dimer were determined by enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Melbourne, Australia), according to manufacturer's instructions. Intra-assay coefficients of variation were below 6.1% and inter-assay coefficients below 6.9%. The minimal detectable dose for TF, TFPI, TAT and D-Dimer were ~4pg/mL, 14.9pg/mL, ~0.5ng/mL and 71pg/mL, respectively. Absorbances were determined using an infinite 200 PRO Spectrophotometer (Tecan Trading AG, Männedorf, Switzerland).

5.4 Statistical Analyses

Sample size was calculated based upon an 11% difference in TFPI from the morning to the afternoon [31], with a power of 0.8 and an α 0.05, 11 participants were required, and 16 were recruited to account for potential dropouts. A Shapiro-Wilk test was used to assess normality of distribution with power output, total time trial completion time, $\%VO_{2max}$ and heart rate data normally distributed, with pre- and post-exercise physiological, perceptual data and markers of haemostasis non-parametrically distributed. All statistical analyses were performed using GraphPad Prism version 5.03 for Windows (GraphPad software, La Jolla California, USA). A one-way ANOVA with repeated measures was used to determine time of day effect within measures of power output, total time trial completion time and $\%VO_{2max}$, with a Bonferroni post hoc test used to determine any significant differences. Pre- and post-exercise physiological and perceptual data, markers of haemostasis and TF to TFPI ratio along with differences in the magnitude of change (post-pre) of haemostasis values were analysed for a time of day effect using a Friedman's Test. A Wilcoxon matched-pairs signed rank test was used to determine differences between pre- and post-exercise physiological, perceptual and blood values for each marker of coagulation and D-Dimer with Cohen's *d* calculated from pre-post blood values (post minus pre, divided by pooled standard deviation). Effect size was interpreted as small <0.2, moderate >0.5 or large >0.8 [271]. All statistical tests were performed at the level of significance of $p<0.05$. Results are expressed as mean ± SD for normally distributed data, with non-parametric data reported as median (range), unless otherwise stated.

5.5 Results

5.5.1 Performance, Physiological and Perceptual Responses to Exercise

Performance, physiological and perceptual responses collected during and immediately post 4km TT at 0830, 1130, 1430, 1730 and 2030 h are presented in **Table 5.1**. No time of day effects were observed in mean power output (p=0.770), TT completion time (p=0.635), heart rate (p=0.677) and RPE (p=0.179). Due to equipment fault with the gas analyser for one participant, n=14 for the measure of $\%V0_{2max}$. Significant main effects for time of day responses was observed for $\%VO_{2max}$ (p=0.037) and effort provided (p=0.030), with $\%VO_{2max}$ greater at 1730 when compared to 2030 h and effort provided higher at 2030 h when compared to 1430 and 1730 h (p=0.017 and p=0.036, respectively).

5.5.2 Pre- and Post-Exercise Physiological Responses

Pre- and post-exercise measures of tympanic temperature, blood lactate and blood pressure were collected at 0830, 1130, 1430, 1730 and 2030 h are presented in **Table 5.2.** A main effect for time of day effects was observed for pre-exercise tympanic temperature (p=0.0003). Post-exercise tympanic temperature was not significantly different across the day (p=0.436). No time of day effects were observed in pre- and post-exercise blood lactate (p=0.945 and p=0.606, respectively), systolic blood pressure (p=0.547 and p=0.542, respectively) and diastolic blood pressure (p=0.950 and p=0.990, respectively).

5.5.3 Exercise and Coagulation Activity

Coagulation responses to the 4km cycling TT when performed at 0830, 1130, 1430, 1730 and 2030 h are presented in **Table 5.3**. Significant increases ($p<0.05$) were observed from pre- to post-exercise for TF, TFPI and TAT at all times of day (0830-2030 h). There was a large post-exercise increase (*d=0.77-1.03*) at 1130 and 1730 h

for TFPI and a moderate to large increase in post-exercise TAT (*d=0.52-0.83*) at 0830, 1130, 1430, 1730 and 2030 h (**Table 5.3**).

5.5.4 Exercise and Fibrinolytic Activity

Originally sixteen participants completed all requirements of the study, however, blood analysis results for fifteen participants are presented due to large outliers in blood results and a subsequent diagnosis of DVT, two months post completion of the present study. Large responses in D-Dimer pre-post exercise were observed for this participant with pre and post 0830 h results 35.1 and 29.5 times greater than the group mean (2250.9 ng/mL and 2653.0 ng/mL, respectively), well above normal values of D-Dimer <500ng/mL [111].

Fibrinolytic responses are presented in **Table 5.3**. Significant differences ($p<0.05$) were observed in pre- to post-exercise D-Dimer across all times of day (0830, 1130, 1430, 1730 and 2030 h).

5.5.5 Coagulation and Fibrinolysis Responses to Time of Day

A significant main effect for time of day for pre-exercise TF ($p=0.004$) and TFPI ($p=0.031$) was observed, with TF greater at 0830 h when compared to 1730 h ($p=0.0007$) and TFPI lower at 1730 h when compared to 2030 h ($p=0.008$). There was no significant effect of time of day responses (0830-2030 h) on pre- TAT ($p=0.364$) and D-Dimer ($p=0.228$). The magnitude of post-exercise change for TF, TFPI, TAT and D-Dimer was not significantly different across the day (TF: $p=0.375$, TFPI: $p=0.236$, TAT: $p=0.873$ and D-Dimer: $p=0.673$, respectively) (**Table 5.3**).

Table 5.1 *Power output (W), total time (m), %VO_{2max} (ml.kg^{-1}.min^{-1}) and heart rate (bpm) measured during each 4km time trial. Ratings of perceived exertion (RPE) and effort were measured upon time trial completion. Data are presented as mean ± SD. + significant difference from 1730 h (p<0.05), *significantly difference from 2030 h (p<0.05).*

	0830	**1130**	**1430**	**1730**	**2030**	**Average**
Power (W)	324.5 ± 34.4	329.5 ± 34.0	327.1 ± 28.2	328.4 ± 31.9	327.6 ± 33.9	327.4 ± 32.5
Total Time (min:sec)	7.16 ± 0.49	7.07 ± 0.50	7.04 ± 0.44	7.08 ± 0.41	7.09 ± 0.42	7.09 ± 0.45
%$V0_{2max}$ (ml^{-1}.kg.min^{-1})	85.5 ± 7.7	84.3 ± 7.6	83.7 ± 7.5	87.3 ± 8.4	$^{+}$82.5 ± 8.4	84.7 ± 7.9
Heart Rate (bpm)	164 ± 10	163 ± 11	162 ± 11	163 ± 13	162 ± 13	163 ± 12
RPE (6-20)	18.4 ± 1.2	18.5 ± 1.6	17.9 ± 2.1	18.2 ± 1.4	18.9 ± 0.8	18.4 ± 1.4
Effort (0-10)	9.1 ± 0.9	9.1 ± 0.9	*8.8 ± 1.0	*8.9 ± 0.8	9.4 ± 0.6	9.0 ± 0.9

Note n= 15 for power, total time, heart rate, RPE, effort; n=14 for %VO_{2max} due to equipment fault with gas analyser for one participant.

Table 5.2 *Tympanic temperature (^{O}C), systolic blood pressure (SBP (mmHg)), diastolic blood pressure (DBP (mmHg)) and blood lactate (Bla ($mmol.L^{-1}$)) measured pre- and post-exercise at 0830, 1130, 1430, 1730 and 2030 h. Data are presented as mean ± SD. *significant difference from 0830 and 1130 h ($p<0.05$), + significant difference from 1430 and 1730 h ($p<0.05$).*

	0830	**1130**	**1430**	**1730**	**2030**
Temp (^{O}C)					
Pre	36.2 ± 1.4	36.8 ± 0.7	*37.3 ± 0.7	*37.3 ± 0.7	+36.9 ± 0.9
Post	36.4 ± 1.1	36.6 ± 1.2	37.9 ± 0.7	37.0 ± 1.1	36.6 ± 1.4
SBP (mmHg)					
Pre	120.9 ± 7	123 ± 10	126 ± 9	124 ± 8	124 ± 6
Post	166 ± 18	164 ± 21	166 ± 19	158 ± 19	166 ± 17
DBP (mmHg)					
Pre	76.8 ± 7.2	77.1 ± 7.2	77.1 ± 5.6	76.6 ± 7.6	78.3 ± 8.4
Post	72.8 ± 9.9	75.0 ± 10.9	74.2 ± 8.5	72.2 ± 11.5	73.9 ± 9.2
BLa ($mmol.L^{-1}$)					
Pre	1.5 ± 0.9	1.5 ± 0.5	1.4 ± 0.7	1.4 ± 0.6	1.6 ± 0.8
Post	11.3 ± 2.4	10.8 ± 3.2	11.3 ± 3.2	10.8 ± 2.6	11.6 ± 3.0

Note n=15

Table 5.3 *Tissue Factor (TF), Tissue Factor Pathway Inhibitor (TFPI), Thrombin Anti-Thrombin Complex (TAT) and D-Dimer and TF:TFPI ratio measured pre- and post-exercise at 0830, 1130, 1430, 1730 and 2030 h. Data are presented as median (range). + significant time of day difference (p<0.05) from 0830 h pre-exercise (TF); @ significant time of day difference (p<0.05) from 1730 h pre-exercise (TFPI); * significant difference (p<0.05) between pre- and post-exercise values. d= effect size.*

	0830		**1130**		**1430**		**1730**		**2030**	
TF (pg/mL)	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**+Pre**	**Post**	**Pre**	**Post**
	5.07 (2.08-71.68)	6.76 (2.35-74.28)	4.57 (1.96-64.14)	5.76 (2.18-64.77)	4.23 (1.70-48.0)	5.86 (2.07-48.34)	4.01 (0.00-50.43)	5.13 (1.86-54.35)	4.25 (0.00-62.50)	4.78 (0.00-67.86)
Δ pre-post	1.18 (0.16-18.38)		0.67 (0.02-9.09)		1.48 (0.34-15.98)		1.34 (0.31-18.71)		0.79 (0.00-15.64)	
p value	.0001*		.0001*		.0001*		.0002*		.0002*	
d	*0.16*		*0.13*		*0.18*		*0.17*		*0.17*	
TFPI (pg/mL)	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**@Pre**	**Post**
	38508 (551-611413)	45249 (11529-78136)	28553 (396-70240))	44808 (958-115657)	33685 (670-65600)	37750 (1016-72125)	14223 (710-73654)	50017 (521-114951)	43281 (710-73654)	50588 (1048-176515)
Δ pre-post	10937 (1306-29927)		16057 (246-45417)		6745 (346-20744)		17194 (67-66507)		9272 (338-102862)	
p value	<.0002*		<.0002*		<.0002*		.0002*		<.0002*	
d	*0.63*		*0.77*		*0.46*		*1.03*		*0.61*	
TAT (ng/mL)	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
	1.69 (0.99-3.03)	2.59 (0.21-4.13)	2.05 (0.35-3.67)	2.39 (1.4-4.04)	1.31 (0.48-4.29)	2.42 (1.19-3.96)	1.85 (0.48-3.50)	2.92 (0.50-4.60)	1.37 (0.49-4.28)	2.16 (1.30-4.40)
Δ pre-post	0.34 (0.07-2.53)		0.41 (0.04-1.60)		0.76 (-0.76-2.7)		0.53 (0.00-1.93)		0.66 (0.09-2.50)	
p value	<.0001*		.0012*		.0002*		<.0001*		.0002*	
d	*0.83*		*0.52*		*0.71*		*0.68*		*0.80*	
D-Dimer (ng/mL)	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
	73.1 (0.0-2250.0)	83.3 (0.0-2653)	52.0 (0.0-1175.0)	137.3 (1.9-1289.0)	47.5 (0.0-1027.0)	128.6 (0.0-9292.0)	29.7 (0.0-1202.0)	123.1 (0.0-2666.0)	64.2 (0.0-1434.0)	121.1 (0.0-593.9)
Δ pre-post	14.1 (-59.6-402.4)		85.3 (1.3-884.1)		51.4 (-20.6-9199.0)		86.5 (-37.5-1464.0)		46.5 (-840.4-210.5)	
p value	<.0001*		<.0001*		.001*		.0001*		.0003*	
d	*0.12*		*0.39*		*0.43*		*0.39*		*0.38*	
TF:TFPI ratio ($x10^2$)	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
	0.011 (0.000-0.282)	0.012 (0.000-0.209)	0.009 (0.000-1.156)	0.007 (0.000-0.152)	0.005 (0.0000-0.264)	0.008 0.001-0.138)	0.004 (0.000-0.719)	0.004 (0.000-0.135)	0.000 (0.000-0.151)	0.003 (0.000-0.134)

n=13 TFPI and TF to TFPI ratio due to removal of outliers

5.6 Discussion

The current study aimed to investigate acute coagulation responses to short-duration high-intensity exercise in a well-trained athletic population, whilst also comparing the responses to exercise performed at several times of the day. The present study is the first, to our knowledge, to investigate diurnal variation in coagulation responses within a well-trained athletic population; with acute coagulation, activation observed regardless of the time of day exercise was performed, as demonstrated by significant increases in post-exercise concentrations of TAT complexes and D-Dimer. Additionally, the results of this investigation also show a time of day response within the pre-exercise measure of TF and TFPI only.

Although activation of blood coagulation as a result of increased blood hypercoagulability post-exercise has been well established [43, 167], the degree of coagulation activation has been demonstrated to be dependent upon numerous factors, with exercise duration and intensity shown to play a key role [40, 49, 50, 166]. Within the present study, when well-trained male cyclists completed a short duration 4km cycling TT, we observed a significant increase in the plasma concentration of TAT complexes (**Table 5.3**), conflicting the findings of previous studies [18, 51]. Indeed, previous research by Hilberg and associates [18, 51] in which short duration (90 s) maximal cycling and exhaustive incremental cycling exercise (mean 19.4 ± 3.4 min) completed by healthy male subjects, demonstrated no significant increases ($p>0.05$) in TAT complexes post exercise, when compared to baseline measures (1.56 ± 0.58 to 1.54 ± 0.46 µg/l and 1.19 ± 0.22 to 1.45 ± 0.42 µg/l , respectively). In contrast to the findings of Hilberg et al. [18, 51] and comparable to the findings of the present study, a significant increase ($p<0.01$) in post-exercise TAT complexes was observed by Dufaux et al. [19] following a maximal incremental test in moderately trained males;

however, the mean duration of the protocol was unreported. This increase in TAT complexes may be due to the exhaustive nature of the exercise protocol, with exhaustive exercise well demonstrated to activate blood coagulation [167]. Indeed, when completing longer duration (1 h) running at intensities ranging from 68%VO_{2max} to 83%VO_{2max} [11], increased TAT complexes were reported to occur at the higher intensity only ($p<0.05$), suggesting that exercise intensity influences thrombin generation. In addition to the intensity (84.7%VO_{2max}) and mean RPE of 18.4 ± 1.4 observed within the present study (**Table 5.1**), our findings suggest a 4km cycling TT to be a high-intensity, high effort bout of exercise, resulting in an increase in hypercoagulability as reflected by an increased TAT complexes post exercise.

Despite no time of day effect observed within pre-exercise measures of TAT and TF to TFPI ratio, higher mean values for both pre- and post-exercise samples of TAT and TF to TFPI ratio (**Table 5.3**) were observed within the morning (0830-1130 h). In addition, a significant main effect for a time of day response was observed for TF ($p=0.004$) with higher values observed at 0830 h when compared to 1730 h, whilst plasma concentrations of TFPI were lower at 1730 h when compared to 2030 h (**Table 5.3**). TF (expressed by endothelial cells and leukocytes[72]), is the primary activator of cell-mediated coagulation, and TFPI is the major physiological regulator of TF-induced coagulation [41]. Taken together, higher TF, TAT and TF to TFPI ratio suggests an overall increase in coagulation activation. This may occur due to an upsurge in haemodynamic forces, increasing laminar shear stress [115], and exposing TF to the blood stream. The exposure of TF results in an increased concentration of TAT complexes [19, 54] and a greater potential for thrombin generation following short-duration high-intensity exercise when completed within the morning (0830 and 1130 h) in well-trained male cyclists. Whilst not statistically different for TAT, this

data suggests that caution should be employed when prescribing short-duration high-intensity exercise in the morning for "at risk athletes" (i.e. older athletes and/or athletes with genetic pre-dispositions to venous thrombosis formation) to reduce the potential for hypercoagulability leading to thrombotic events to occur.

Activation of the coagulation system following short duration high-intensity exercise is accompanied by simultaneous activation of fibrinolysis [42, 98, 116, 175, 272]. Similar to the findings of elevated D-Dimer concentrations ($p<0.01$) following moderate intensity exercise as reported by Molz et al. [14], significant elevations in post-exercise concentrations of D-Dimer (an *in vivo* marker of fibrin degradation) were reported within the present study following all five 4km cycling time trials (0830-2030 h) (**Table 5.3**). When investigating the activation of the fibrinolytic system in response to exercise, previous studies [6, 9, 10, 37] have failed to disclose the time of day in which the exercise protocols were conducted, thus time of day effects on post-exercise markers are rarely reported. The findings of the present study demonstrates that when completing a short-duration high-intensity TT at 0830, 1130, 1430, 1730 and 2030 h, a time of day influence is present in the post-exercise activation of the fibrinolytic system occurs regardless of the time of day, corresponding with the reported increase in the activation of the coagulation system.

Diurnal variations within markers of coagulation and fibrinolysis at rest have been reported previously, with increased hypercoagulability and hypofibrinolysis observed between morning and noon when compared with other times of the day [7, 25-27, 31, 45, 188, 190]. In contrast to previous studies [7, 31, 273], we observed no significant time of day effects in pre-exercise markers of TAT spanning from 0830-2030 h; however, a main effect for time of day responses was observed for TF and TFPI (**Table 5.3**). When investigating healthy men and women in the morning (0700-1000 h) and

afternoon (1500-2100 h), an absence of diurnal variations within TAT, TF and D-Dimer are not unusual and have been previously reported by Jafri et al. [190] and Deguchi et al. [136]. Unlike TF, TAT and D-Dimer, diurnal variations of TFPI levels have been reported [31, 45]. Indeed, Pinotti et al. [31] observed a significant difference in TFPI activity with higher values in the morning (0800 h) when compared with the afternoon (1400 h) within healthy male participants. In contrast to the findings of Pinotti et al. [31], we observed a significant difference in pre-exercise TFPI activity, with the highest concentration observed at 2030 h and not in the morning (**Table 5.3**). This may be due to an increased concentration of TF pre-exercise at 2030 h; however, no significant differences observed within pre-exercise physiological measures (**Table 5.2**) might explain why the increase in TF has occurred.

Previous research has demonstrated morning surges to occur within blood pressure [115]. This places an increased laminar shear stress on the endothelium lining the blood vessels, which may account for an increase in TF, TFPI and TAT within the morning (**Table 5.3**), as demonstrated within the current study. We observed no significant differences in blood pressure (**Table 5.2**). Whilst blood pressure was not significantly different, pre-exercise tympanic temperature showed a significant time of day effect ($p=0.0003$), with significant responses observed between 0830 and 1130 h when compared with 1430 and 1730 h ($p<0.05$) and between 1430, 1730 and 2030 h TT ($p<0.02$) (**Table 5.2**). This is not uncommon, with core body temperature previously shown to peak between 1600 and 2000 h [266]. In an investigation into the effect of time of day on platelets, Aldemir et al. [7] reported higher evening core body temperature contributed to temperature-induced vasodilation, enhancing platelet release. However, the influence of body temperature on markers of coagulation and fibrinolysis is unknown and further research investigating this potential relationship is

therefore required. With elite and well-trained athletes required to compete in different environmental conditions for both national and international competitions, this potential relationship may have important implications for athlete's overall health and well-being along with performance outcomes.

The risk of athletes developing a venous thrombosis is relatively low (1 in 1000 people [44]) and is similar to that of the general population [33]. However, athletes are exposed to many acquired "athlete-specific" risk factors associated with hypercoagulability (i.e. long-haul travel, trauma, immobilisation after injury, haemoconcentration after exertion, and polycythaemia) [3] that may be enhanced when combined with a pre-existing conditions such as inheritable clotting disorders (i.e. Factor V Leiden or Prothrombin G20210A mutations) [32, 46, 47]. Whilst the results of the present study show acute activation of the coagulation and fibrinolytic systems regardless of time of day in well-trained cyclists, caution should be applied when prescribing short-duration, high-intensity exercise at different times of the day for "at risk athletes", however, further research within these populations is therefore required. These includes the investigation of older athletes (such as master level athletes who may travel long haul to competitions) and particularly individuals with pre-existing acquired and/or genetic pre-dispositions to clotting.

5.7 Conclusion

In conclusion, a short-duration high-intensity exercise bout results in acute activation of the coagulation and fibrinolytic systems, regardless of the time of day the exercise task is performed, within this selected group of participants only. A time of day effect was observed within the pre-exercise measure of TF at 0830 h and TFPI at 2030 h, but not within other markers of coagulation (TAT) and fibrinolysis (D-Dimer); however, higher mean values for TAT were observed at 1130 h. Therefore, even in a well-

trained population, caution should be applied when completing short-duration high-intensity exercise between 0830-1130 h. Whether these findings are applicable for older athletes, clinical populations and those with acquired or genetic predisposition to clotting requires further investigation.

Chapter 6: Compression socks and the effects on coagulation and fibrinolytic activation during marathon running

This manuscript has been accepted as an original research investigation in the European Journal of Applied Physiology (Accepted June 30th, 2018) and will appear in the literature as:

Zadow, E.K., Adams, M.J., Wu, S.S.X, Kitic, C.M., Singh, I., Kundur, A., Bost, N., Johnston, A.N.B., Crilly, J., Bulmer, A.C., Halson, S.L. & Fell. J.W. "Compressions socks and the effects on coagulation and fibrinolytic activation during marathon running". *European Journal of Applied Physiology.*

Journal Impact Factor: 2.130

Rationale

Whilst study three (chapter four) demonstrated the ability of a short-duration high-intensity exercise bout to activate coagulation, the ability to attenuate haemostatic responses within exercise has rarely been investigated. With marathon running well demonstrated to activate the haemostatic system and marathons typically completed in the morning (when hypercoagulability is at its greatest, as observed in study three), study four provided a convenient opportunity to test whether the use of compression clothing would reduce haemostatic activation in athletes.

6.1 Abstract

Purpose: Compression socks are frequently used in the treatment and prevention of lower-limb pathologies; however, when combined with endurance-based exercise, the impact of compression socks on haemostatic activation remains unclear. ***Objectives:*** To investigate the effect of wearing compression socks on coagulation and fibrinolysis following a marathon. ***Methods:*** Sixty-seven participants (43 males (mean±SD: age: 46.7±10.3 y) and 24 females (age: 40.0±11.0 y), were allocated into a compression (SOCK, n= 34) or a control (CONTROL, n=33) group. Venous blood samples were obtained 24h prior to and immediately post-marathon, and were analysed for thrombin anti-thrombin complex (TAT), tissue factor (TF), tissue factor pathway inhibitor (TFPI), and D-Dimer. ***Results:*** Compression significantly attenuated the post-exercise increase in D-Dimer compared to the control group (median (range) SOCK: +9.02, -0.34 to 60.7 ng/mL, CONTROL: +25.48, (0.95 to 73.24 ng/mL). TF increased following the marathon run (median (range), SOCK: +1.19 (-7.47 to 9.11) pg/mL, CONTROL: +3.47, (-5.01 to 38.56) pg/mL in all runners. No significant post-exercise changes were observed for TAT and TFPI ($p>0.05$). ***Conclusions:*** Whilst activation of coagulation and fibrinolysis was apparent in all runners post-marathon, wearing compression socks was shown to reduce fibrinolytic activity, as demonstrated by lower D-Dimer concentrations. Compression may reduce exercise-associated haemostatic activation when completing prolonged exercise.

Key words: Compression, d-dimer, tissue factor, tissue factor pathway inhibitor, thrombin-anithrombin complexes

6.2 Introduction

Whilst physical activity and exercise appear to protect against thromboembolic episodes [40], exercise has long been recognised to induce transient activation in blood coagulation [10], platelet aggregation [12] and fibrinolytic activity [14], increasing the potential for deep vein thrombosis (DVT). The degree to which these systems are activated are often dependent upon the duration and intensity of the exercise, together with the study population investigated [15]. Evidence for the activation of the coagulation system following strenuous endurance exercise (i.e., marathon distance, 42.2 kilometres) are reflected by specific markers of *in-vivo* thrombin generation such as thrombin anti-thrombin complexes (TAT) [10, 152] and prothrombin fragment 1+2 [20, 94, 95]. TAT has been widely used as a valuable marker of activated blood coagulation during exercise, with significant increases in TAT reported immediately post-marathon [10, 95, 152], and following endurance exercise of shorter duration [50, 94].

The fibrinolytic system is activated in unison with the coagulation system, presumably to preserve haemostatic balance [152]. This has been demonstrated by elevated measures of D-Dimer, a marker of *in-vivo* fibrin degradation, in addition to TAT, immediately upon marathon completion [10, 16]. However, the regulation and activation of haemostasis is complex and involves many factors other than TAT and D-Dimer.

Whilst 1 in 1000 athletes will experience a post-exercise thromboembolic event [2, 44], exercise-induced haemostatic activation may not be detrimental to most participants. However, the risk of an adverse event is compounded by underlying and acquired risk factors associated with hypercoagulability such as oral contraceptive use and long-haul travel, and dehydration [274]. Several case studies documenting the

occurrence of deep vein thrombosis in otherwise healthy athletes following endurance based exercise have been documented [2, 38], highlighting the potentially detrimental effects of athletic training that may in the worst case scenario, result in a fatality.

Compression socks are frequently used for the treatment and prevention of lower extremity clinical pathologies, including DVT, with compression socks shown to maintain the coagulation and fibrinolytic balance through increases in both venous and arterial blood flow [223]. The benefits of clinical compression garments have been inferred by the sporting clothing industry, and their use continues to grow in popularity due to their proposed enhancement of exercise performance and recovery [227, 228], despite a lack of clear scientific evidence of their effects on physiology. The impact of compression socks on haemostatic activation during endurance exercise has rarely been investigated, with only one previous study with a small sample size (n=20) reporting a decrease in overall haemostatic activation following a marathon when compression socks were worn [24]. It was hypothesized that when worn during a marathon, compression socks will reduce haemostatic activation.The aim of this study was to investigate the effect of compression socks on exercise-induced activation of the coagulation and fibrinolytic systems when worn during prolonged endurance exercise.

6.3 Methods

6.3.1 Participants

Forty-three males (mean ± SD: age: 46.7 ± 10.3 y, height: 1.80 ± 0.1 m, body mass: 77.7 ± 13.1 kg) and 24 females (age: 40.0 ± 11.0 y, height: 1.60 ± 0.1 m, body mass: 58.0 ± 9.3 kg) were recruited to participate in the study through an email sent to all registered marathon runners (n=6216), with runners also recruited the day before the 2016 Gold Coast Marathon (Gold Coast, Queensland, Australia) at the Gold Coast

Exhibition Centre. All consenting study participants (recruited via email and the day prior to the marathon) were screened by the primary researcher to ensure all participants were non-smokers, with no known history or clinical signs of metabolic conditions or coagulation disorders. Participants were excluded from the study if they had a previous history of thromboembolism. Female participants were excluded if they were taking the oral contraceptive pill, with all participants required to be illness and injury free, whilst avoiding the use of anticoagulant (i.e., aspirin, heparin, and warfarin) and non-steroidal anti-inflammatory medications for at least two weeks before, and throughout the testing period. Furthermore, participants were to avoid the use of all types of compression garments whilst travelling to the Gold Coast and in the lead up to the marathon itself. Participants were provided with a written description of the risks and benefits of the study and provided signed informed consent prior to their inclusion within the study. Details that may disclose the identity of the subjects have been omitted. All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

6.3.2 Study Overview

This was a randomised control trial investigating the effects of compression socks on strenuous exercise-induced activation of coagulation and fibrinolysis. All participants recruited were randomly assigned using a computer generated code to either the control (CONTROL, 21 males and 12 females (n=33)) or the compression sock (SOCK, 22 males and 12 females (n=34)) group. Foot-to-knee compression socks were provided by 2XU (24/7 Compression Socks, 2XU North America LLC, Carlsbad, CA, USA) to participants within the SOCK group, with sock size dependent upon shoe size and self-reported lower leg circumference.

6.3.3 Marathon Characteristics

The marathon began at 07:30 h and was completed on a flat terrain with no elevation, at an average temperature of 16.4 ± 4.3^{O}C and 69.0 ± 20.4% relative humidity.

6.3.4(1) PRE-Marathon Venous Blood Sample

Twenty-four hours prior to the marathon, all participants reported to the Gold Coast Exhibition Centre between 10:00-14:00 h for the collection of a PRE-marathon venous blood sample. During this visit, participants within the SOCK group received their compression socks and were instructed to wear their socks to the marathon, during the marathon and following the marathon until the POST-marathon blood draw (immediately POST: ~10-20 min). Participants within the CONTROL group were instructed to refrain from wearing any type of compression garment prior to the first venous blood sample (PRE-marathon), and throughout the duration of the marathon and the recovery period (i.e. marathon completion until post-marathon venous blood sample collection).

6.3.4(2) Post-Marathon Venous Blood Sample

A second venous blood sample was collected immediately within 10-20 min upon completion of the marathon (POST) in a medical tent, less than 100m from the finish line between ~10:00- 14:00 h.

6.3.5 Blood Sample Collection

Blood samples were drawn without stasis by venepuncture from the antecubital fossa from participants in a supine position. Blood samples for determining markers of coagulation and fibrinolysis were collected into 3.8% sodium citrate Vacutainers® (Becton Dickinson, New Jersey, USA). Within four hours of collection, citrated blood

samples were centrifuged at room temperature for 15 min at 2,000 g. Plasma supernatant samples were aliquoted and stored at -80^{o}C until required for analysis.

6.3.6 Blood Sample Analyses

Tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombin anti-thrombin complexes (TAT) and D-Dimer were determined by enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Melbourne, Australia) according to manufacturer's instructions. Inter-assay coefficients of variation were below 6.2% and Intra-assay coefficients were below 5.9%. The minmal detectable dose for TF, TFPI, TAT and D-Dimer were ~4pg/mL, 14.9pg/mL, ~0.5ng/mL and 71pg/mL, respectively. All samples were measured in duplicate. Absorbance for each marker was read using an infinite 200 PRO Spectrophotometer (Tecan Trading, AG, Männedorf, Switzerland). Changes in plasma volume were calculated for TF, TFPI, TAT and D-Dimer as described in the literature according to the method of Dill and Costill [275].

6.3.7 Statistical Analyses

A Shapiro-Wilk test was used to assess normality of distribution, with all markers of haemostasis (TAT, TF, TFPI and D-Dimer) non-parametrically distributed. All statistical analyses were performed using GraphPad Prism version 5.03 for Windows (GraphPad software, La Jolla California, USA). Baseline participant characteristics (age, weight, BMI, training volume), overall marathon finishing time, differences between groups in PRE- and POST-marathon measures of haemostasis (TAT, TF, TFPI and D-Dimer), along with differences in the magnitude of change (PRE-POST), were analysed using a Mann-Whitney U Test. Cohen's *e*ffect sizes (*d*) were calculated from PRE-POST blood values within and between conditions (CONTROL vs SOCK), calculated as $d_{NP} = Z/\sqrt{N}$ where $_{NP}$ is non-parametric, Z is the z-score calculated from Wilcoxon Signed Rank test and N is the number of participants, and interpreted as

small <0.2, moderate >0.5 or large >0.8 [271]. Results are presented as median (range), unless otherwise stated.

6.4 Results

Participant Characteristics

There were no significant differences in baseline characteristics between groups for age, mass, body mass index (BMI) and training volume (h.wk^{1}) (all p>0.05) (**Table 6.1**).

Coagulation and Fibrinolytic Responses

PRE- and POST- marathon coagulation and fibrinolytic results for CONTROL and SOCK groups are presented in **Table 6.2**. Plasma concentrations of TAT, TF, TFPI and D-Dimer did not differ between groups at baseline (p=0.328, p=0.063, p=0.278 and p=0.807, respectively). Significant increases in PRE- to POST-marathon measures of TF and D-Dimer were observed in both the CONTROL (p=0.001 and p=0.001, respectively) and SOCK group (p=0.040 and p=0.001, respectively), whilst no significant changes in TAT and TFPI were observed (all p>0.27) (**Table 6.2**). The magnitude of post-exercise increase in D-Dimer was smaller in the SOCK group (9.03,-0.34 to 60.8 ng/mL) compared to the CONTROL group (25.5, 0.95 to 73.2 ng/mL; p=0.008, d_{NP}=0.43) (**Figure 6.1**). The difference between the CONTROL and SOCK groups in the magnitude of post-exercise change was small for TAT, TFPI and TF (all d_{NP} <0.24) (**Table 6.2)**.

Marathon Finish Time

No significant differences (p=0.106) were observed in official marathon finishing times when comparing the SOCK and CONTROL groups (4:29:23 ± 1:17:19 and 4:27:26± 1:16:15, respectively).

6.5 Discussion

The aim of this study was to investigate the effect of compression socks on strenuous exercise-induced activation of the coagulation and fibrinolytic systems of haemostasis following a marathon. Wearing compression socks during a marathon attenuated the post-exercise increase in D-Dimer concentration. In addition, acute activation of coagulation and fibrinolysis were observed immediately POST-marathon, as reflected by significant increases in plasma concentrations of TF and D-Dimer, regardless of whether SOCKS were worn.

Within the present study, and in line with previous research [20, 152, 180], marathon running was associated with elevated concentrations of D-Dimer. Indeed, our findings are similar to those of Siegel et al. [180] in which a significant increase in post-marathon concentrations of D-Dimer (177 ± 137 to 529 ± 279 ng/mL; $p<0.001$) were observed within 4 h of marathon completion. When worn during a marathon, compression socks were shown to reduce the magnitude of increase in POST-marathon concentrations of D-Dimer (**Figure 6.1**), contrasting the findings of Zaleski et al. [24] in which concentrations of D-Dimer did not differ when compression socks were worn versus not worn at any given time point (i.e. pre-, post- +24 h post-marathon). However, Zaleksi et al. [24] failed to present the pre- and post-marathon data for D-Dimer itself; therefore, these findings should be interpreted with caution. Compression socks and pneumatic calf compression devices appear to increase fibrinolytic activity [22] through inhibiting components associated with Virchow's Triad (i.e. venous stasis, hypercoagulability and vessel wall damage), ultimately reducing the risk of thrombosis formation. Indeed, in a case study by Zaleski et al. [229] plasma concentrations of D-Dimer were reported to be ~30% lower in a single runner with a genetic pre-disposition to blood clotting (i.e. F5 1691 A risk allele,

Factor V Leiden mutation) when compression socks were worn versus not worn over two marathon runs. Therefore, when worn during a marathon, compression socks may reduce the potential for clot formation, as indicated by reduced D-Dimer (clot fibrinolysis) formation. However, research on the use of compression socks to maintain the balance of both the coagulation and fibrinolytic systems during prolonged strenuous exercise is limited; therefore, further investigations are required to verify the findings of the present study and those of Zaleski et al. [24, 229]

In agreement with previous research [94, 95, 152], simultaneous activation of the coagulation system was observed in all participants as indicated by significant increases in TF (the primary activator of the TF-coagulation pathway [272]), regardless of whether compression socks were worn (**Table 6.2**). The present findings are in contrast to those of Weiss et al. [77] who failed to demonstrate a significant increase in post-exercise concentrations of TF (205 ± 43 to 218 ± 47 pg/mL; $p>0.05$, $d=0.29$) albeit in a shorter duration (1 h) maximal run, suggesting the duration of the exercise itself may be a factor in exercise-induced activation of coagulation. In addition, when comparing the magnitude of change between the CONTROL and SOCK group, a non-significant difference with a small ES was observed within plasma concentrations of TF (**Figure 6.1**), suggesting compression socks do not "dampen" the activation of the coagulation system when completing a marathon run. However, in order to confirm these findings, further research investigating a more extensive range of well-known markers of coagulation activation are therefore required.

When combined with muscular activity, compression socks reduce venous stasis, whilst increasing the volume and velocity of blood flow in the deep venous system, resulting in decreased blood pooling in the distal calf veins (i.e. posterior tibial vein,

anterior tibial vein, and peroneal vein), reducing haemostatic activation and increasing fibrinolytic potential [223, 229]. Indeed, with a greater range of participants wearing compression socks (n=34) versus a control group (n=33) within the present study, our findings are in contrast with those of Zaleski et al. [24], who reported post-marathon plasma concentrations of TAT to be 17% lower (p=0.07) in runners assigned to the sock group when compared to the control group. In addition, when worn during the 2013 Hartford marathon, compression socks were shown to reduce the magnitude of change in post-marathon concentrations of TAT (*d*= 3.00). Several published studies have demonstrated significant post-marathon increases in plasma concentrations of TAT [10, 95, 152]; however, this was not the case within the present study despite similar runner characteristics (age, training volume and marathon finish times), blood collection and sample analyses procedures. The absence of a post-exercise increase in TAT within the CONTROL and SOCK groups is unusual, however our findings are similar to those of Parker et al. [152] in which no significant increases in TAT were observed post-marathon within a control group, when investigating the effects of travel and marathon running combined. However, Parker et al. [152] found a significant increase in TAT was observed after the marathon in the runners that had travelled more than four hours to compete (TRAVEL: 5.0 ± 4.0 to 12.9 ± 15.6 µg/L vs CONTROL: 4.0 ± 1.2 to 6.1 ± 1.2 µg/L; p=0.04, *d*=0.81). Therefore the impact of air-travel in combination with endurance-based exercise, is of particular interest, with an upsurge in national and international participation in varying duration endurance-based events (i.e. triathlons, marathons and Iron Man competitions), highlighting the need for further research, especially with an aging population.

When completing prolonged strenuous exercise (i.e. 42.2km marathon), runners are exposed to ‘running-specific’ risk factors increasing the risk for venous thrombosis

generation through repetitive micro-trauma resulting in foot strike haemolysis, dehydration and endothelial damage [274, 276]. Increased thrombosis risk may be a direct result of an upsurge in haemodynamic forces, increasing laminar shear stress [115], and exposing TF to the blood stream, initiating the coagulation cascade and resulting in an increased concentration of TAT complexes [54]. Indeed, an increase in markers indicative of tendency for coagulation activation following the marathon was observed in both the CONTROL and SOCK group, as demonstrated by the relatively similar effect sizes (CONTROL, small to moderate and SOCK, small to large, respectively) for plasma concentrations of TF. Therefore, within the present study, coagulation and fibrinolytic activation was observed within both the CONTROL and SOCK group, with lowered activation observed within the runners assigned to the SOCK group.

6.6 Practical Applications

Whilst compression socks are frequently used within the sporting industry to enhance exercise performance and aid recovery, the use of compression socks within endurance-based exercise, may reduce the potential for thrombosis development. When compression socks are not worn when completing a marathon run, our results demonstrate an increase in plasma concentrations of D-Dimer, suggesting an increased potential for thrombin generation. Therefore, the use of compression socks when completing a marathon run may benefit endurance-based athletes. To date, investigations into the use of compression socks and haemostatic activation in prolonged strenuous exercise is limited, thus further research is required to confirm the findings of the present study and those of Zaleski et al. [24]

6.7 Limitations

This study was not without limitations. Whilst participants were excluded from participating if they had a known and previous history of thromboembolism, participants were not screened for genetic mutations for thrombophilia including Factor V Leiden mutation. The influence of travel on markers of haemostasis were not controlled for within the present investigation, however, previous investigations have identified that air travel augments haemostatic activation immediately following a marathon [102, 152].

6.8 Conclusions

Whilst activation of coagulation and fibrinolysis was apparent in all runners following the marathon, our results suggest an increase in fibrinolytic activity as reflected by an increase in D-Dimer, when compression socks were not worn throughout a marathon run. Therefore, compression socks may assist in the reduction of exercise-associated coagulation and fibrinolytic activation when completing prolonged strenuous exercise, however, further research is required make firm conclusions regarding the beneficial effects of compression socks.

Table 6.1 *Mean (± SD) baseline measures of physical and performance characteristics for the SOCK versus CONTROL participants.*

Variable	**CONTROL (n=33)**	**SOCK (n=34)**	**P value**
Age (years)	45.2 ±11.5	43.5 ±10.5	0.662
Weight (kilograms)	69.9 ±15.6	71.4 ±14.9	0.512
Body mass index ($kg.m^{-2}$)	23.2 ±3.5	23.8 ±3.1	0.379
Training volume (hour:min:wk^{-1})	5:02 ±2:13	4:39 ±1:58	0.147
Official finishing time (hour:min:secs)	4:27:26 ± 1:16:15	4:29:23 ±1:17:19	0.106

Table 6.2 *Thrombin Anti-Thrombin Complex (TAT), Tissue Factor (TF), Tissue Factor Pathway Inhibitor (TFPI), D-Dimer measured pre- and post-marathon for the CONTROL and SOCK group. Data are presented as median (range). * significant difference between pre- and post-marathon values within group. dNP = effect size.*

	CONTROL (n=33)				SOCK (n=34)			
	PRE	**POST**	**P value**	d_{NP}	**PRE**	**POST**	**P value**	d_{NP}
TAT (ng/mL)	3.19 (0.16 to 6.28)	3.09 (0.56 to 7.31)	0.272	0.14	2.47 (0.34 to7.31)	2.48 (0.99 to 7.78)	0.400	0.10
TF (pg/mL)	3.86 (0.00 to 5.53)	7.41 (2.16 to 42.5)	<0.001*	0.54	5.06 (0.13 to 14.2)	5.84 (0.84 to 17.1)	0.040*	0.26
TFPI (ng/mL)	24.85 (0.29 to 77.33)	22.70 (0.28 to 88.50)	0.840	0.03	17.9 (0.50 to 48.5)	17.3 (0.41 to 72.6)	0.490	0.09
D-Dimer (ng/mL)	5.22 (0.00 to 24.14)	33.71 (5.27 to 88.00)	<0.001*	0.70	5.50 (0.05 to 33.3)	16.7 (1.02 to 78.2)	<0.001*	0.43

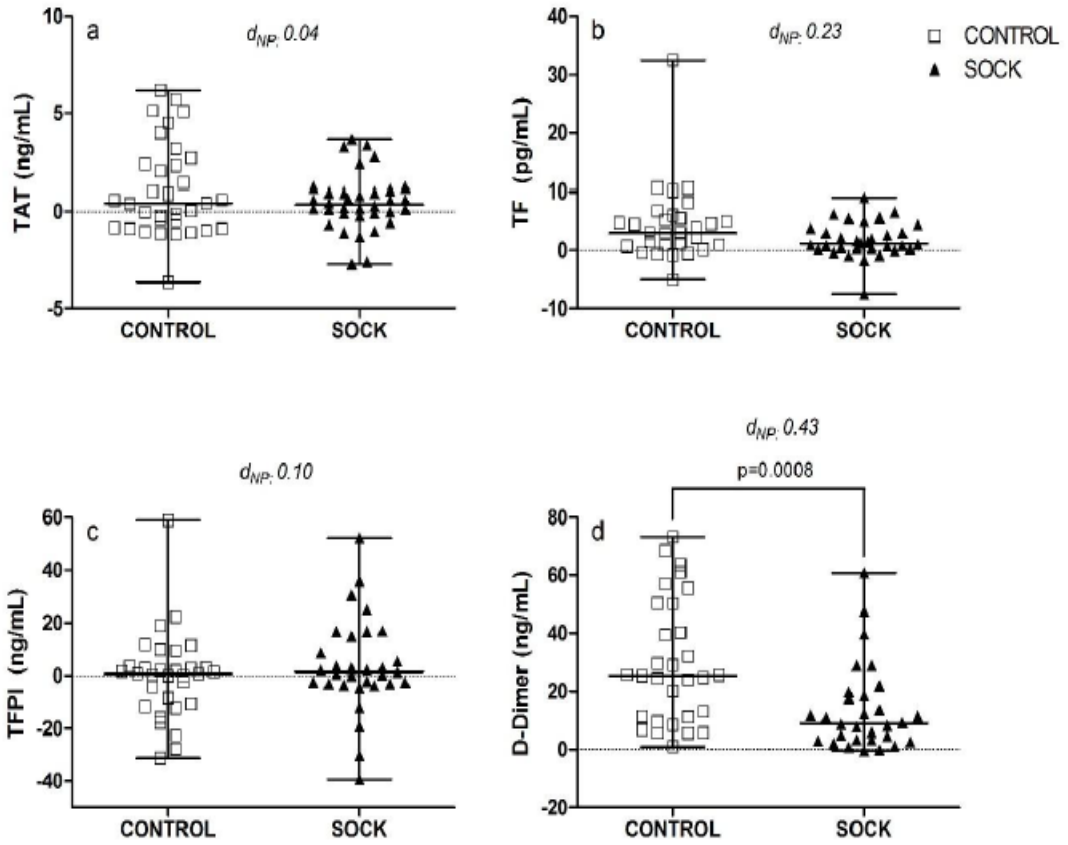


Figure 6.1 *Median (range) magnitude of change (Δ) between PRE-POST measures of a) Thrombin Anti-Thrombin complexes (TAT), b) Tissue Factor (TF), c) Tissue Factor Pathway Inhibitor (TFPI), and d) D-Dimer for the CONTROL and SOCK group. dNP=effect size.*

Chapter Seven: Discussion

The overarching aim of this thesis was to investigate factors that may influence the overall haemostatic response, including activation of the coagulation and fibrinolytic systems, in trained and well-trained athletes. The activation of these systems may lead to the development of VTE. Yet known risk factors for VTE specific to an athletic population have previously been examined in untrained healthy populations only [7-9]. As such, results from these studies may not be directly applicable to an athletic population. To help further understand these risk factors and their influence on haemostasis in trained athletes, four studies were undertaken during this PhD. Better understanding of these factors may reduce the potential for the onset and development of VTE, resulting in the maintenance of overall athlete health and well-being whilst minimising detrimental performance outcomes.

The major outcomes from this thesis are:

i) the KICKR is a valid ergometer for measuring cycling power output,

ii) a 4km cycling TT, when completed on the KICKR, is a reliable performance test for trained cyclists,

iii) a short-duration high-intensity TT significantly activates the coagulation and fibrinolytic systems in well-trained cyclists, regardless of the time of day,

iv) TF and TFPI only are influenced by time of day, suggesting an increased activation of coagulation to occur in the morning, and

v) when worn during a marathon, compression socks reduce exercise-associated fibrinolytic activity as reflected by lower D-Dimer concentrations.

Study one (chapter three) is the first investigation to assess the validity of power output of the Wahoo KICKR Power Trainer (KICKR: a cycling ergometer enabling cyclists to use their own bicycles) using a dynamic calibration rig (CALRIG). Ergometer errors of <2% are required to detect meaningful changes in performance [237, 258] with the KICKR demonstrated to provide valid measures of power output, possessing a small mean bias and narrow limits of agreement of (-1.1% (95%LoA: -3.6 to 1.4%)) when assessed over 250-700W at 80-120rpm.

These findings for the KICKR are consistent with the ergometer errors of the Velotron and Schoberer Rad Messetechnik (SRM) power meters (<1%) when compared with a CALRIG [234]. When cyclists' own bicycles are used, the KICKR possesses a smaller ergometer error than the PowerTap (1.8%) [277], the Axiom PowerTrainer (2.2%) [278], the SRM (2.3%) [239] and the Ergomo Pro (2.3-4.1%) [279], respectively. Conversely, at the lower and higher ranges of power output (100-200W and 750-999W), larger mean biases outside of the acceptable range for ergometer error [237] were observed, with the findings for the KICKR similar to those of Gardener and colleagues [239] for other power measurement methods.

To investigate the scientific rigor of the KICKR for performance testing, study two (chapter four) examined the reliability of a 4km cycling TT when performed on the KICKR, using trained cyclists. The 4km TT was proven to be highly reliable for the measurement of power output only, with a coefficient of variation (CV) of 3.4%, falling below the required CV of 5% [243]. Additionally, the average power in which the TTs were performed (344 ± 41W) fell within the validated range of power when performed on the KICKR (250-700W (study one, chapter three)). Thus, the 4km TT was demonstrated to be a reliable measure of performance when performed on the KICKR and suitable for research purposes implemented within study three.

Study three (chapter five) employed both the KICKR and a 4km TT to investigate acute changes in the activation of coagulation and fibrinolysis following short-duration high-intensity exercise at different times of the day (0830, 1130, 1430, 1730 and 2030 h) in well-trained cyclists. Activation of the coagulation system as demonstrated by significant post-exercise increases in TAT complexes have been reported in long duration exercise bouts (>20 min) [19, 50]. However, a lack of consensus and published research exists as to whether coagulation is activated in shorter duration exercise bouts of less than 20 min. When completing a 4km cycling TT (mean duration 7.09 ± 0.45 min) using well-trained cyclists, significant activation of the coagulation system (as demonstrated by higher post-exercise TAT concentrations) was observed.

The elevated post-exercise TAT in study three is in contrast to previous research investigating coagulation activation via plasma concentrations of TAT in healthy trained populations undertaking exercise bouts of less than 20 minutes [18, 51] (**Figure 7.1**). Hilberg and associates found no significant increases ($p>0.05$) in TAT when compared to baseline measures upon immediate completion of 90 s maximal isokinetic cycling (1.56 ± 0.58 to 1.54 ± 0.46 µg/l; $p>0.05$) [18] or a maximal incremental cycling test of mean duration: 19.4 ± 3.4 min (1.30 ± 0.20 to 1.45 ± 0.42 µg/l) [51]. The absence of a post-exercise increase in TAT complexes may be due to factors such as the training status of the individuals investigated, with training status demonstrated to attenuate post-exercise increases in TAT [280]. However, with relatively similar VO_{2max} observed within study three (60.3 ± 8.1ml.kg^{-1}.min^{-1}) and the studies of Hilberg and associates (56.0 ± 8.1ml.kg^{-1}.min^{-1}) [18] and (59.1 ± 6.5ml.kg^{-1}.min^{-1}) [51], this is unlikely to account for the significant increase in post-exercise TAT observed when completing a 4km TT. These differences might better be

explained by the amount of time spent above a critical exercise intensity for activation of coagulation pathways. Indeed, when completing longer-duration (1 h) running at intensities ranging from 68%VO_{2max} to 83%VO_{2max} [11], higher concentrations of TAT were observed at 83%VO_{2max} only ($p<0.05$), suggesting the length of time spent at a sustained higher intensity influences thrombin generation. Similarly, Hilberg and associates [281] found a 33% increase in post-exercise thrombin generation (as reflected by TAT complexes) following 60 to 90 min of sustained cycling at 90% individual anaerobic threshold.

The findings within the studies of Weiss et al. [11] and Hilberg et al. [281] highlight the importance of not only the intensity in which exercise is performed, but the duration that the intensity is maintained, as key factors influencing coagulation activation. Interestingly, whilst Hilberg et al [51] and Dufaux et al. [19] implemented similar exercise protocols (maximal incremental cycling tests), Dufaux and associates [19] reported significantly higher concentrations of TAT after the exercise. These differing findings may be a result of a longer duration maximal exercise test (~23 min) in the study of Dufaux and colleagues [19], with participants possibly spending a greater amount of time at higher exercise intensities (~5-10 min) than the participants in the study by Hilberg et al. [51]. The post-exercise increase in TAT complexes observed within study three after approximately seven minutes of intense exercise (85%VO_{2max}) is comparable to the findings of Dufaux and associates [19]. Therefore, in order to observe exercise-induced coagulation activation, exercise duration may require exercise intesnities between or above 83-85%VO_{2max} and approaching seven minutes duration to elicit significant changes in TAT. The findings of study three suggests that a 4km TT is both long enough and when performed at an intensity high enough, results in activation of the coagulation system.

It is well established that haemostatic markers are subject to circadian rhythms when measured under resting conditions, with hypercoagulability and hypofibrinolysis observed to occur between 0600 and 1200 h [7, 25, 26, 188, 190]. However, this has only been investigated in untrained and sedentary individuals. Therefore, study three aimed to investigate if time of day effects were present within pre-exercise concentrations of TAT, TF, TFPI and D-Dimer in well-trained cyclists. Despite no statistically significant time of day effects observed for TAT or D-Dimer ($p>0.2$), a significant main effect for time of day was observed for TF ($p=0.004$), and TFPI ($p=0.031$). Study three is the first to my knowledge to demonstrate a time of day effect in TF (in the absence of exercise) (**Figure 7.1**), and may be a direct result of an upsurge in haemodynamic forces [115], exposing TF to the blood stream and promoting thrombin generation at 0830 h, supporting the notion of morning hypercoagulability.

Diurnal variations of TFPI levels have been previously reported [31, 45], with higher values observed in the morning (0800 h), when compared to the afternoon (1400 h) in healthy male participants [31]. The findings of study three are in contrast to those of Pinotti et al. [31], with study three reporting higher TFPI concentrations observed at 2030 h (median (range): 43,281 (710 to 73,654 pg/mL)) compared to 0830 h (median (range): 38,508 (551 to 61,143 pg/mL)). The higher pre-exercise TFPI at 2030 h in study three may be linked to the higher (but not significantly) pre-exercise concentrations of TF and D-Dimer also observed at 2030 h. Whilst the majority of thromboembolic events peak within the morning [28], a secondary peak has been reported to occur between 1800 to 2000 h [282], which may provide a potential explanation for the higher concentrations of TFPI, TF and D-Dimer at 2030 h. Additionally, Iversen et al. [26] previously demonstrated D-Dimer to peak at both 0900 and 2300 h (when measured under resting conditions), which may have resulted

in the increased concentration of TFPI as a compensatory mechanism to the increased concentrations of TF and D-Dimer. However, this remains speculative with no conclusive evidence. Therefore, further investigations are required to confirm a secondary peak in coagulation and fibrinolytic activation within a well-trained population.

The findings of study three provide valuable insight into haemostatic responses within a well-trained population, when completing a short-duration, high-intensity bout of exercise. No time of day effects were observed for the exercise-induced activation of coagulation and fibrinolysis, with activation occurring to a similar magnitude regardless of the time of day exercise was performed (**Figure 7.1**). However, study three found a time of day effect within the pre-exercise measures of TF and TFPI, but not within the markers of TAT and D-Dimer, a novel finding for the markers and study population assessed (**Figure 7.1**). Whether these findings are applicable to older athletes and those with acquired or genetic predispositions to clotting remains unclear, highlighting the need for further examination.

Whilst study three demonstrated the ability of a short-duration high-intensity exercise bout to activate coagulation, the ability to attenuate haemostatic responses to exercise bouts has rarely been investigated. Although compression socks are recommended to wear during long-haul travel to diminish the risk of VTE [36], whether compression socks reduce the activation of haemostasis when worn during exercise is relatively scarce [24, 283]. Therefore, study four (chapter six) aimed to investigate the effect of compression socks on the activation of haemostasis following a marathon run (42.2km).

Whilst frequently used within the sporting industry to enhance exercise performance and recovery, only a single study on the impact of compression socks on haemostatic activation during endurance-based exercise [24] has been published alongside a single case study [229]. When worn during a marathon run in study four, compression socks were shown to reduce the magnitude of increase in concentrations of D-Dimer, contrasting the findings of Zaleski and associates [24] (**Figure 7.1**), who found concentrations of D-Dimer did not differ between the sock wearing group versus the control group at any given time points (pre-, post-, +24 h post-marathon). The lack of significant difference observed for D-Dimer between the sock and control group may be due to the relatively small sample size (n=20 total (n=10 sock, n=10 control)) potentially masking the true response of D-Dimer to marathon running. Compression socks appear to decrease fibrinolytic activity [22] through the inhibition of components associated with Virchow's Triad (i.e. reduced venous stasis, hypercoagulability and vessel wall damage), which may account for the attenuation of D-Dimer levels within runners assigned to the compression sock group.

In a case study by Zaleski et al. [229], plasma concentrations of D-Dimer were reported to be ~30% lower in a runner with a genetic predisposition to blood clotting (F5 1691 A risk allele, Factor V Leiden mutation) when compression socks were worn versus not worn over two marathon runs. Similar to the findings of study four, when worn during a marathon compression socks may reduce the potential for clot formation as indicated by reduced D-Dimer formation (**Figure 7.1**). However, there is a paucity of research into the use of compression socks to maintain the balance of both the coagulation and fibrinolytic systems during prolonged strenuous exercise (**Figure 7.1**), with further investigations required to confirm the findings of study four and those of Zaleski and associates [24, 229].

In agreement with previous research [94, 95, 152], study four demonstrated some activation of the coagulation system in all participants as indicated by significant increases in TF post-marathon [272], regardless of whether compression socks were worn. However, these findings for TF were not evident with regards to TAT complexes and in contrast to those of Zaleski et al. [24] who found a significant post-marathon increase in TAT complexes, with average TAT concentrations reported to be lower and approaching significance ($p=0.07$) in runners wearing compression socks. The differences between study four and Zaleski et al. [24] for TAT may be linked to the interaction between intensity and duration (as proposed within study three as a key factor for thrombin generation) and the sample size investigated.

The argument for an intensity and duration relationship may be supported by the differences in mean marathon finish times, with the runners in the study of Zaleski et al. [24] completing the marathon in an overall faster time of 4h 11min 38sec and therefore at a potentially higher exercise intensity, when compared to the finish time of 4h 28min 23 sec for participants in study four. Additionally, and as previously mentioned, the findings of Zaleski et al. [24] for TAT may be a type 1 error, due to the research potentially being underpowered as evident from the small sample size of only 20 participants. Based upon these findings, a standard marathon run does not elicit post-exercise increases in TAT complexes due to the low intensities marathons are typically completed at, with previous research within this field underpowered (**Figure 7.1**). Future research should investigate the relationship between TAT and overall intensity when undertaking marathon running.

Alongside activation of the coagulation system, marathon running was also demonstrated to increase fibrinolytic activity. Therefore, this study has demonstrated that compression socks may assist in the reduction of exercise-associated fibrinolytic

activation when completing prolonged marathon running (**Figure 7.1**). However, further research is required to make firm conclusions regarding the beneficial effects of compression socks on haemostatic responses, especially in athletes who are travelling long distances to the event, older athletes and those with acquired or genetic predispositions to hypercoagulability.

7.1. Directions for future research

Identified as a valid cycling ergometer (study one) and a reliable measure of performance (study two), the KICKR and a 4km cycling TT was deemed suitable for research purposes within study three. Whether TTs of varying distances are reliable when completed on the KICKR is yet to be determined. Therefore, further investigation into the reliability of longer duration TTs when completed on the KICKR will assist researchers in the correct selection of a measure of performance when investigating haemostatic responses to exercise of varying distances and durations.

No time of day response for coagulation and fibrinolytic activation was observed when completing a short-duration high-intensity exercise bout in study three. The short duration of the exercise bout itself may have potentially masked the influence of time of day on the coagulation and fibrinolytic systems; however, whether a longer duration TT when completed at different times of the day will influence haemostatic responses is yet to be determined. Further research is therefore required to clarify if longer duration TTs will reveal a time of day influence on haemostatic responses to exercise in well-trained athletes.

The activation of the coagulation and fibrinolytic systems upon completion of a 4km TT was confirmed in study three, regardless of the time of day exercise was performed. Whether master level athletes and athletes with genetic predispositions to

hypercoagulability respond in a similar manner to the findings of study three remains unclear, with research currently lacking within this population. Thus, it is of interest to understand the acute haemostatic responses to exercise of short-duration and high-intensity within these populations and in particular if the time of day in which the exercise task is performed influences the activation of the haemostatic system. The outcomes of this research may assist with the implementation of preventative strategies including external mechanical prophylaxis to minimise the risk of potentially adverse outcomes (i.e. VTE).

Whilst acute activation of the coagulation and fibrinolytic systems were demonstrated in exercise with a mean duration of 7 min (study three), whether these findings are directly transferable to well-trained athletes from other sports comprising of similar-duration high-intensity events (i.e. 3000 m run, 800 m swim), has yet to be investigated. With the exercise mode often responsible for the variability observed within previous research [9, 112, 284], the investigation of haemostatic responses to differing modes of exercise remains unclear with a lack of research investigating this variable. Future research should focus on the examination of haemostatic responses to exercise of varying modes within a well-trained population in an attempt to contribute to this area of research. These findings may assist with exercise mode prescription to reduce the risk and onset of VTE in athletes returning to exercise following injury.

When worn during a marathon run, compression socks reduced fibrinolytic activity in healthy runners (study four). Whether similar haemostatic responses are present when compression socks are worn during a marathon within athletes of: varying fitness levels, ages and genetic predispositions to clotting remains unconfirmed. More research is required to clarify the haemostatic responses within these populations of

athletes to determine if compression socks may be used as an effective preventative measure.

Additionally, the combined effects of travel, compression garments and marathon running on haemostatic activation warrants further investigation, with only a limited number of studies completed within this field [24, 152, 283]. Limitations within these previous studies have included smaller sample sizes, in addition to the failure of the authors to provide detailed methodology making interpretation of findings difficult. Further investigations are therefore required using healthy and well-trained marathon runners and standardised study protocols to adequately assess haemostatic responses to travel, compression garments and exercise. Additionally, the influence of travel and marathon running have failed to collect post-travel venous blood samples upon arrival to the destination with future research required to collect pre-travel, immediately post-travel and pre-exercise venous blood samples. These post-travel blood samples are crucial in the evaluation of coagulation and fibrinolytic markers (more specifically, D-Dimer) to determine if elevated markers pre-exercise may increase the risk for thrombosis when combined with endurance-based exercise. These findings will be of benefit to athletes undertaking travel via the implementation of VTE preventative measures including: the use of compression garments or short-term anticoagulant therapy, in order to compete either professionally or recreationally.

7.2. Conclusion

The overall outcomes from this series of studies demonstrate that factors including the duration of exercise influence coagulation responses within a well-trained population, findings previously limited to apparently healthy and untrained populations only (**Figure 7.1**). In addition, this thesis contributes to the scarce literature available on the use of compression garments on haemostatic responses in endurance-based

exercise, establishing that the use of sports compression socks may be beneficial in reducing the risk of VTE. The outcomes of this thesis may assist coaches, sports scientists and the athletes themselves to consider preventative measures for VTE, especially in athletes deemed to be most “at risk”.

7.3 Practical Applications

Based upon the findings of studies 3 and 4, some practical/ clinical applications may be relevant. For example, due to a time of day effect observed within TF, peaking within the morning (0830 h), caution should be applied when prescribing short-duration high-intensity exercise bouts at 0830 h, especially in athletes who are predisposed to hypercoagulability (i.e. genetic thrombophilia, increasing age). Whilst compression socks are frequently used within the sporting industry to enhance exercise performance and aid recovery, the use of compression socks within endurance-based exercise may reduce the potential for thrombosis development. When compression socks are not worn when completing a marathon run, our results from study 4 demonstrate an increase in plasma concentrations of D-Dimer, suggesting an increased potential for thrombin generation. Therefore, the use of compression socks and haemostatic activation in prolonged strenuous exercise is limited, thus further research is required to confirm the findings of the present study and those of Zaleski et al. [24]

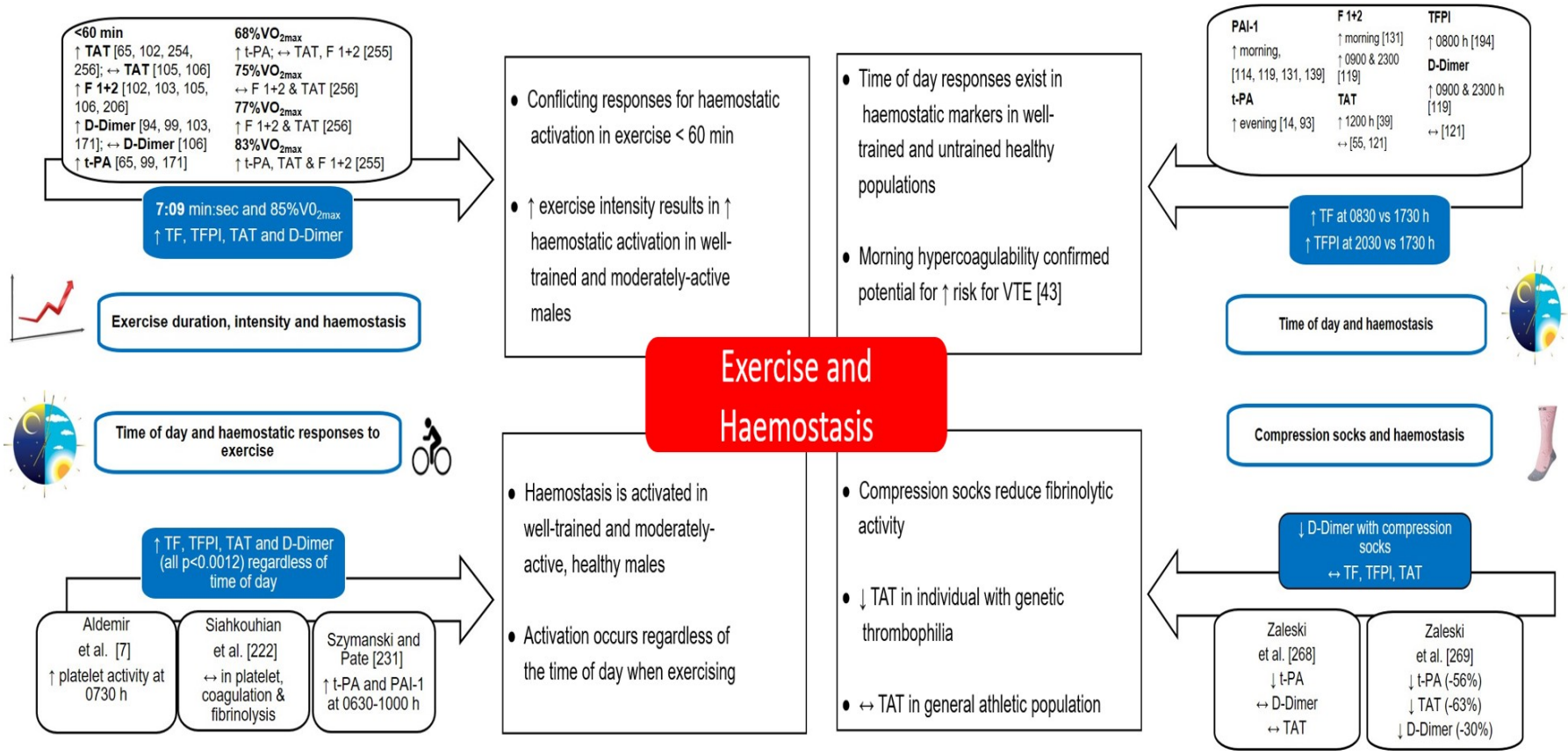


Figure 7.1 *Schematic representation of the overall outcomes and key findings of this PhD (indicated in blue boxes) in relation to exercise and haemostasis, when compared to previous research (white boxes).*

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Appendices

Appendix A: "Reliability of power settings of the Wahoo KICKR Power Trainer after 60 hours of use" Manuscript

An original version of this chapter has been published in the International Journal of Sports Physiology and Performance as a brief review and appears in the literature as:

Zadow, E.K., Kitic, C.M., Wu, S.S.X. & Fell, J.W. "Reliability of power settings of the Wahoo KICKR Power Trainer after 60 hours of use". *International Journal of Sports Physiology and Performance.* 2018 Jan 1;13(1): 119-121 doi: 10.1123/ijspp.2016-0732; Epub 2018 Jan 11.

Journal Impact Factor: 2.654

Abstract

Purpose: The purpose of this study was to assess the reliability of power output measurements of a Wahoo KICKR Power Trainer (KICKR) on two separate occasions, separated by fourteen months of regular use (~1 h per week). ***Methods:*** Using the KICKR to set power outputs, powers of 100-600W in increments of 50W were assessed at cadences of 80, 90 and 100rpm which were controlled and validated by a dynamic calibration rig (CALRIG). ***Results:*** A small mean bias of 0.8% (95%LoA: -8.3-8.9%) was observed over 100-600W at 80-100rpm between Trial 1 and Trial 2. Small mean biases with acceptable limits of agreement were observed at 80rpm (1.2%, 95%LoA: -5.9-8.3%), 90rpm (0.1%, 95%LoA: -7.8-5.5%) and 100rpm (1.0%, 95%LoA: -1.6-3.6%). Intraclass correlation coefficients (ICC) with 95% confidence intervals (CI) for mean power (W) between trials was 1.00 (95%CI: 1.00-1.00) with a typical error (TE) of 3.1W and 1.6% observed between Trial 1 and Trial 2. ***Conclusion:*** When assessed at two separate time points fourteen months apart, the KICKR has acceptable reliability for combined power outputs of 100-600W at 80-100rpm, reporting overall small mean biases with acceptable limits of agreement and low TE. Coaches and sports scientists should feel confident in the power generated by the KICKR over an extended period of time when performing laboratory training and performance assessments.

Keywords: reproducibility, power, ergometry, training

Introduction

Advancements in the technology of cycling ergometers have been shown to provide increasing accuracy in the measurement of power output. The measurement of power throughout cycling is one of the key determinants of performance and is vital for evaluating individual differences in performances, monitoring the effectiveness of both training/ergogenic aids, whilst providing a true representation of the performance capabilities of both recreational and elite cyclists.[1] Ergometers which enable the use of cyclists own bicycles have been shown to produce reliable results predictive of competitive performance whilst replicating movement economy and enhancing ecological validity in the transfer of power from a laboratory setting to the field.[2] Therefore, the ability of a cycling ergometer to consistently record reliable measures of power output with a high degree of precision is of significant importance.

It is important for coaches and sports scientists to ensure the power measuring device they are using as part of research/athlete support is reliable.[3] To track meaningful changes in competitive performance from an ergogenic or training intervention in elite athletes, ergometer error/bias should be less than 2%.[4] However, the majority of validity and reliability assessments on cycling ergometers have been assessed over individual occasions,[5,6] with evidence suggesting older ergometers may show greater biases when reliability is investigated over an extended period of time.[7,8] With changes in performance as small as 1% often determining the difference between a finish on the podium or within the peloton, the ability to continuously track and monitor changes in performance on the same piece of equipment over an extended period of time is therefore required.

The Wahoo KICKR Power Trainer (KICKR: Wahoo Fitness, Atlanta, GA) allows for the attachment of cyclists' own bicycles and has previously been shown to provide

valid measures of power output,[9] falling within the manufacturers claims of accuracy (± 3%). However, whether the Wahoo KICKR Power Trainer continues to provide continuous reliability over an extended period after regular use (i.e., research, performance assessments and training) has yet to be investigated. Therefore, the purpose of this study was to assess the reliability of power output measurements of the Wahoo KICKR Power Trainer on two separate occasions, separated by fourteen months of regular use.

Methods

The reliability of the KICKR power output was assessed on two separate occasions, fourteen months apart after ~120 experimental time trials (~60 h duration), by comparison with the power output of a dynamic calibration rig (CALRIG: Flinders University, Dynamic Calibrator 34118, Adelaide, Australia) as previously described.[5,9] Both assessments of power were conducted in standard laboratory conditions (18.5 ± 3.5°C and 41.5 ± 3.5% relative humidity).

A bicycle was attached to the KICKR via the SRAM/Shimano cassette. Power outputs of 100-600W were achieved by manually varying the resistance settings within 'ergometer-mode' within the Wahoo Fitness Application for the KICKR (Wahoo Fitness, 2014 (version 5.1.1)). Power was increased by 50W every 3 min at fixed cadences of 80, 90 and 100rpm. Power measured by the CALRIG was sampled at 200Hz and recorded every second (1Hz), with data produced in the final minute of each stage used for analysis purposes, according to the methodology of Hopker et al.[6] A standardised twenty-minute cool down was provided between each assessment cadence.

Statistical Analysis

Sustained reliability of power from the KICKR was determined using bias and 95% limits of agreement (LoA) in accordance with the methods of Bland and Altman.[10] The relative error (%) in measurement of power was calculated by subtracting the power measured by the CALRIG in Trial 2 from the power measured by the CALRIG in Trial 1, divided by the average measured power of Trial 1 and Trial 2. The absolute error (difference in watts) was calculated by subtracting the CALRIG Trial 2 power from CALRIG Trial 1 power with all data analysed using GraphPad Prism 5, version 5.03 (La Jolla, CA, USA). In accordance with previous research, [5] relative measurement bias of <1.5%, 1.5-2.5% and >2.5% were deemed as highly reliable, moderately reliable and inaccurate, respectively. Using an Excel spreadsheet for reliability,[11] intraclass correlation coefficient (ICC) in combination with 95% confidence intervals (CI), was used to determine the degree of association between the logarithmically transformed recorded CALRIG power at Trial 1 and Trial 2. Absolute (W) and relative (%) typical errors (TE) were calculated by dividing the standard deviation of the difference between Trial 1 and 2 by the square root of 2.

Results

Overall, small relative (%) and absolute biases (W) of 0.8% (95%LoA: -3.7-5.3%) and 0.3W (95%LoA: -8.3-8.9W), respectively, were observed between trials over 100-600W and 80-100rpm. Biases of 1.2% (95%LoA: -5.9-8.3%); 0.1% (95%LoA: -7.8-5.5%); and 1.0% (95%LoA: -1.6-3.6%) with absolutes biases of 0.2W (95%LoA: -9.9-10.3W); -1.1W (95%LoA: -2.2-2.4W) and 1.8W (95%LoA: -6.9-10.4W) were observed over 80, 90 and 100rpm and 100-600W, respectively. At the lower ranges of power (100-200W) over 80-100rpm, biases for relative (3.1% (95%LoA: 3.0-9.2%)) and absolute (4.0W (95%LoA: -2.5-10.6W)) measures of power, were larger than the

relative and absolute biases of 0.2% (95%LoA: -1.7-2.1%) and 0.3W (95%LoA: -6.1-6.7W) observed between 250-500W over 80-100rpm.

Average relative error (%) in measures of power from the CALRIG between trials over 100-600W and 80-100rpm are presented in **Figure 1**. Errors >2.5% were observed at the lower ranges of power (100-150W) at 80rpm with moderate errors for 90rpm also observed at 100-150W. Moderate errors at 100rpm were reported for power ranging from 100-350W with overall power from 200-600W at 80-100rpm demonstrated to be highly accurate, with relative errors of <1.5% reported.

An ICC with 95% CI of 1.00 (95%CI: 1.00-1.00) was observed for mean power reported by the CALRIG between Trials 1 and 2, using the KICKR over 100-600W and 80-100rpm. The Bland-Altman plot (**Figure 2**) clearly illustrates the average absolute differences in recorded power across the same range and cadence. There was a between trial typical error of 3.1W and 1.6%.

Discussion

The purpose of the current study was to examine the reliability of power measurements provided by the Wahoo KICKR Power Trainer on two separate occasions, fourteen months apart. The main findings from the study suggests the KICKR to have high test-retest reliability, falling within the recommended range for ergometer error, with low typical errors reported for measures of power output over an extended period of regular use.

The accuracy of an ergometer to record reliable measures of power over an extended period of time is of importance, with ergometer errors of <2% required for monitoring changes in cycling performance.[4] Whether ergometer error remains low over extended periods of time is rarely investigated, with only one previous study to have reported

this.[7] On two separate occasions 11 months apart, Gardner et al.[7] reported similar reproducibility (~2.5% error) between the SRM and PowerTap mobile power trainers, suggesting good long-term reliability, however, falling outside the recommended range for ergometer error.[4] In contrast to the findings of Gardner et al.,[7] the KICKR was shown to have high test-retest reliability when compared to the CALRIG on two separate occasions, falling within the recommended range for ergometer error, with an overall small mean bias of 0.8% and a low typical error of 1.6%. Similar to Hopker et al.[6] and Gardner et al.,[7] we observed greater discrepancies within the lower ranges of power outputs (100-200W), with an overall large mean bias of 3.1% (95%LoA:-3.0-9.2%) and 4.0W (95%LoA:-2.5-10.6W), with average relative errors of 5.1, 3.0 and 1.3%, respectively, at cadences of 80-100rpm (**Figure 1**). This discrepancy may have arisen from a systematic error/consistent bias in the reading of power provided by the KICKR at the lower ranges of power, regardless of cadence.[4]

Despite large biases at the lower ranges of power, a small bias of 0.2% (95%LoA: -1.7-2.1%) was reported for power ranging from 250-500W, falling within the manufacturers claims for accuracy (± 3%). With mean power for competitive and well-trained cyclists observed to consistently exceed 250W,[12] this falls within the acceptable range of ergometer error for the KICKR (250-500W and 80-100rpm). However, as previously reported,[9] accuracy of power measures of the KICKR are influenced by cadence selection.

When assessed over 100-600W for Trial 1 and 2, small biases of 1.2% (95%LoA: -5.9-8.3%), 0.1% (95%LoA: -7.8-5.5%) and 1.0% (95%LoA:-1.6-3.6%) were reported for 80, 90 and 100rpm, respectively. However, when assessed at 100rpm, a mean error of 2.1% was observed for powers of 100-350W (**Figure 1**). Regardless, with the KICKR falling within the acceptable range for ergometer error as reported by

Hopkins,[4] coaches and sports scientists should feel confident in the reliability of power generated by the KICKR over an extended period, enabling accurate monitoring of performance changes.

Conclusions

In conclusion, when assessed at two separate time points fourteen months apart, the results of the present study suggest that the Wahoo KICKR Power Trainer has acceptable reliability for combined power outputs of 100-600W at 80-100rpm, falling within the recommended range for ergometer error. Coaches and sports scientists should feel confident in the power generated by the KICKR over an extended period of time following regular use when performing laboratory training, performance assessments and for talent identification purposes.

Acknowledgements

The results of the present study do not constitute endorsement of the product by the authors or by the journal. The authors would like to thank the Tasmanian Institute of Sport for access to the CALRIG, Mrs Alanna Martin, Mr John Gregory and Mr Stephen Stone for technical assistance and Mr Taylor Wilczynski and Mr Torben Partridge-Madsen for assistance with data collection. At the time of this study, the corresponding author was the recipient of an Australian government funded Postgraduate Research Scholarship (Australian Postgraduate Award).

Table A.1 *Ratio bias and 95%rLoA for differences in recordings pf Power at 100-600W at 80-100rev.min^{-1}*

Power (W)	Ratio bias (95%rLoa)
100-600	1.002 (0.992-1.011)
100-150	1.009 (0.993-1.024)
100-200	1.007 (0.993-1.020)
250-500	1.000 (0.997-1.004)

KICKR Set Power (Watts)	CALRIG Set Cadence (rpm) 80	90	100	Average (%)
100	10.6	2.0	2.5	5.1
150	5.5	1.7	1.9	3.0
200	0.7	1.1	2.1	1.3
250	0.2	0.2	2.5	0.9
300	0.0	0.7	1.5	0.7
350	-0.6	-1.3	1.9	0.0
400	-0.4	-0.9	-0.2	-0.5
450	-0.3	-0.1	0.9	0.2
500	-0.8	-0.2	0.3	-0.2
550	-0.9	-1.3	-0.5	-0.9
600	-0.8	-0.8	-1.4	-1.0

Figure A.1. *Relative error (%) between dynamic calibration rig (CALRIG) measured power at Trial 1 and Trial 2 over a set power range of 100-600W using the Wahoo KICKR Power Trainer (KICKR), and cadences of 80-100rev.min^{-1}. Errors of <1.5%, 1.5-2.5% and >2.5% are colour coded as green, yellow and red.*

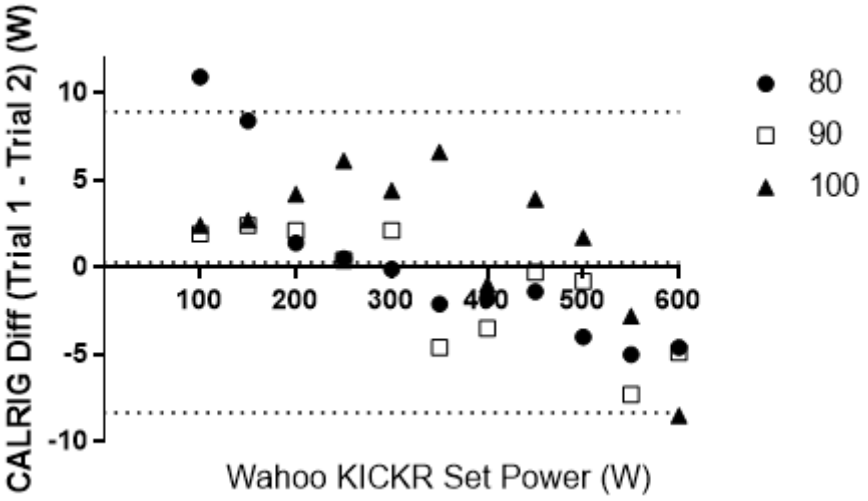

Figure A.2. *Bland-Altman plot of the mean difference in absolute power output (W) between Trial 1 and Trial 2 over 100-600W as recorded by the Wahoo KICKR Power Trainer (KICKR) and the dynamic calibration rig (CALRIG) over 80-100rev.min^{-1}.*

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Appendix A, i: "Sustained Reliability of the Wahoo KICKR Power Trainer over an extended period" Conference Poster

UNIVERSITY of TASMANIA

Sustained reliability of the Wahoo KICKR Power Trainer over an extended period

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Introduction

- The reliability of a cycling ergometer to consistently record reliable measures of power outputs is of significant importance.
- To detect meaningful changes within competitive performance over prolonged periods of time, ergometers should provide accurate measures of power with no evidence of drift regardless of time frame between assessments, its age and frequency of use.
- This study assessed the reliability of power measurements of the Wahoo KICKR Power Trainer (KICKR) on two separate occasions, separated by more than one year using a dynamic calibration rig (CALRIG).

Methods

- On two separate occasions 14 months apart, power settings produced by the KICKR were compared to the power measured by a CALRIG in standard laboratory conditions (18° C and 40% RH).
- Power outputs of **100-600 W** (50 W increments) were controlled by the KICKR (in ergometer mode) at cadences of **80, 90 and 100 rpm** controlled by the CALRIG.
- Agreement between measured power values was assessed using a Bland-Altman analysis (mean bias with 95% Limits of Agreement [LoA]) and calculation of the typical error (TE).

Results

- Small differences in recorded power outputs were observed between trial 1 and trial 2 with a mean bias of 0.3 W (95%LoA: -8.3-8.9 W, **Figure 1**). Mean differences between the two trials at cadences of 80 rpm, 90 rpm and 100 rpm were small with biases of 0.2 W, -1.1 W and 1.8 W with acceptable limits of agreement at 80 (95%LoA: -9.8-10.2 W), 90 (95%LoA: -7.8-5.5 W) and 100 rpm (95%LoA: -6.8-10.4 W).
- A small bias of 0.3 W (95%LoA: -6.0-6.7 W) between trial 1 and trial 2 was observed between 250-500 W over 80-100 rpm (**Figure 2**).
- A typical error of 3.1 W was observed between trial 1 and trial 2.

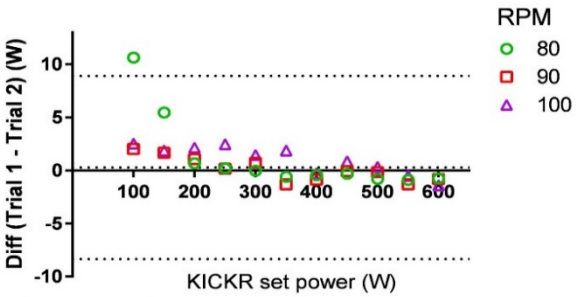

Figure 1. *Bland-Altman plot of the difference in measured power output (W) between Trial 1 and Trial 2 using the KICKR at 100-600 W and cadences of ◦ 80rpm, □ 90rpm and Δ 100rpm. Dashed lines represent the mean bias and 95% Limits of Agreement.*

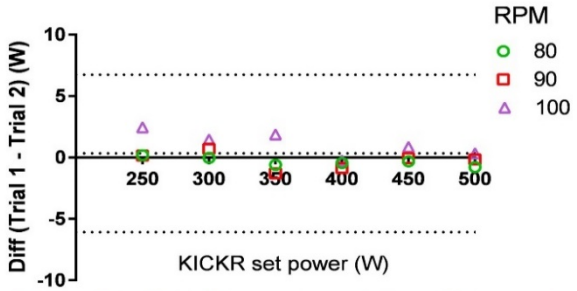

Figure 2. *Bland-Altman plot of the difference in measured power output (W) between Trial 1 and Trial 2 using the KICKR at 250-500 W and cadences of ◦ 80rpm, □ 90rpm and Δ 100rpm. Dashed lines represent the mean bias and 95% Limits of Agreement.*

Conclusion

- When assessed at two separate time points 14 months apart, the KICKR has acceptable reliability for combined power outputs of 100-600 W at 80-100 rpm, reporting overall small mean biases with acceptable limits of agreement and low TE. However, caution should be applied over 100-150 W at 80 rpm, with larger errors observed.
- Coaches and sports scientists should feel confident when monitoring performance changes using the KICKR between 250-500 W over 80-100 rpm (power typically observed within sustained aerobic efforts) over an extended period of time.

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Appendix Bi: Ethics Approval

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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

10 March 2015

Dr James Fell
School of Health Sciences, Faculty of Health
University of Tasmania
Building C Newnham Campus
Locked Bag 1322
Launceston TAS 7250

Sent via email

Dear Dr Fell

REF NO: H0014346
TITLE: Does Time of Day influence Pacing Selection in a 4km Time Trial?

Document	***Version***	***Date***
Consent Form	V1	7 August 2014
Consent Form	V2	3 October 2014
NEAF - Original		12 February 2015
NEAF – Revised- signed		
Research Plan - Time of Day		14 July 2014
Time of Day advertisement flier-	V1	4 August 2014
Time of Day participant information sheet	V1	6 August 2014

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation on **21 October 2014** to be conducted at the following site(s):

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the Health and Medical HREC. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on the Ethical Conduct in Human Research* (NHMRC 2007 updated 2014).

Therefore, the Chief Investigator's responsibility is to ensure that:

(1) The individual researcher's protocol complies with the HREC approved protocol.

(2) Modifications to the protocol do not proceed until **approval** is obtained in writing from the HREC. Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.

(3) Section 5.5.3 of the National Statement states:

Researchers have a significant responsibility in monitoring approved research as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies and take prompt steps to deal with any unexpected risks.

The appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested. http://www.research.utas.edu.au/human_ethics/medical_forms.htm

(4) All research participants must be provided with the current Patient Information Sheet and Consent Form, unless otherwise approved by the Committee.

(5) The Committee is notified if any investigators are added to, or cease involvement with, the project.

(6) This study has approval for four years contingent upon annual review. A *Progress Report* is to be provided on the anniversary date of your approval. Your first report is due **21 October 2015.** You will be sent a courtesy reminder closer to this due date.

(7) A *Final Report* and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact me on (03) 6226 2764.

Yours sincerely

Digitally signed by Lynda Hobman
DN: cn=Lynda Hobman, o=University of Tasmania, ou=Office of Research Services, Research Integrity & Ethics, email=lynda.hobman@utas.edu.au, c=AU
Date: 2015.03.10 11:03:41 +11'00'

Lynda Hobman
Administration Officer (Integrity and Ethics)
Research Integrity and Ethics Unit
Office of Research Services
University of Tasmania
Private Bag 01
Hobart Tas 7001
T +61 3 6226 6254

Appendix B. ii: Participant Information Sheet

UNIVERSITY of TASMANIA | FACULTY OF HEALTH

Project Title: Does Time of Day Influence Pacing Selection in a 4 km Time Trial?

Investigators Dr. James Fell
Dr. Cecilia Shing
Dr. Chris Abbiss
Dr. Jeremiah Peiffer
Ms. Emma Zadow

Location School of Health Sciences
Locked Bag 1320
University of Tasmania
Launceston, Tasmania 7250

Contact Miss Emma Zadow

Emma.Zadow@utas.edu.au

Invitation

You are invited to participate in this study as you are:
1. Male aged between 18-50 years
2. Minimum of 2 years cycling experience
3. Have previous experience with time trials
4. Are willing to visit the University of Tasmania's Exercise Physiology Laboratory on campus over seven separate occasions for a total of ~ 7.5 hours

This study is being conducted in partial fulfilment of a PhD for Emma Zadow under the supervision of Dr James Fell and Dr Cecilia Shing, from the School of Human Life Sciences at the University of Tasmania, with Dr Chris Abbiss (Edith Cowan University, WA) and Dr Jeremiah Peiffer (Murdoch University, WA).

Introduction

With exercise shown to be beneficial for overall health and wellbeing, it has been well reported that the duration and intensity of the exercise itself may lead to a transient increase in the activation of the coagulation system, resulting in an increased risk for thrombosis (i.e. blood clot) development. The markers associated with coagulation have been shown to possess diurnal variations, peaking within the hours of the early morning (08:00 to 12:00

noon) and decreasing as the day progresses (12:00 noon onwards). However, the time of day in which this research is conducted is rarely reported.

The ability to pace during competitive cycling performance is of significant importance with cyclist's selection of pacing directly influencing their overall performance. The time of day has been shown to impact athletic performance with a 10% increase in performance results observed when competing between the hours of 16:00 to 20:00 h (known as an athlete's peak circadian window). Upon comparison of morning and evening performances, significantly slower morning performances have been widely reported, with greater time to exhaustion reported within evening trials.

With vast information identifying diurnal variations within coagulation and performance, the influence of time of day within short bouts of exercise on pacing selection and coagulation responses are yet to be examined. Therefore, the purpose of this study will look to examine the influence of time of day at five different time points on the coagulation response to exercise and pacing strategy selection during a 4 km performance time trial.

Participants

We require 15 highly- trained male cyclists. Cyclists will have a minimum of two years of competitive cycling and time trial experience. Cyclists must avoid any strenuous exercise 24 hours prior to testing. As a possible participant in this study, you will be asked to visit the University of Tasmania's Exercise Physiology Laboratory on seven separate occasions with no less than two and no greater than seven days between visits. Each visit would require approximately one hour to complete with your total participation estimated at 7 hours.

During your initial visit, you will complete a maximal exercise test on your own bicycle attached to the Wahoo Kickr Power Trainer. This test will start at a light resistance and increase in resistance each minute until you are no longer able to continue (usually 8 – 20 minutes). Throughout the test, you will have your expired breath and heart rate collected. This data will be used to determine your aerobic fitness level and maximal power output.

The second visit will be used to familiarise you with each component of the study protocol and the equipment used throughout your subsequent trials. During this session, you will complete a 4 km time trial, aiming to complete this trial in as short as time as possible.

During visits three to seven, identical methodologies to the second visit will be implemented with the only difference being the time of day the trial is completed (i.e. 08:30, 11:30, 14:30, 15:30, 18:30 h).

Blood Collection
Throughout this study, you will be asked to provide two venous blood samples (pre- and post-exercise) during each of the five experimental exercise sessions (does not include the maximal exercise test or the first familiarisation session) for a total of ten samples. After each blood draw, you will be provided with the opportunity, if needed, to rest up to 30 minutes before starting any exercise.

Physiological and Perceptual Measurements
Other measurements collected throughout the scope of this study will include tympanic temperature, heart rate and expired ventilatory gases, collected throughout the duration of you participation (i.e. warm up and experimental protocol component), with your blood pressure collected pre- and post- experimental trials. Motivation and mood scales will also be presented within the form of a written questionnaire and will be required to be completed once again, pre- and post- experimental session over the five prescribed trials. These blood, physiological and perceptual measurements are an important part of this study as they will allow measurement of circadian rhythm influence at a physiological level; thus it is necessary that you are prepared to provide all ten samples

Voluntary Participation
It is important that you understand that your involvement in this study is voluntary and we will respect your right to decline. There will be no consequences to you if you decide not to participate, and this will not affect your treatment/ service. If you decide to discontinue participation at any time, you may do so without providing an explanation. If you withdraw, all information you have provided will be destroyed. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. All of the research will be kept on a password protected computer of the primary researcher.

Possible Benefits
Participants who complete this study will be provided with information such as maximal aerobic power and oxygen consumption, both which can be used to help the development of training programs. Furthermore, participants will be provided with information regarding the most appropriate time of day to complete their training in order to enhance cycling time trial performance.

Possible Risks
All five experimental trials will require participants to complete a 4 km time trial in as short as time as possible. The intensity of these time trials will be similar to that experienced during a race, which all athletes will be familiar with. In rare circumstances, it is possible that individuals could become light- headed and/or nauseous after completing such events. It is important to note that if you do become light headed and/ or nauseous, you will not be

allowed to leave the facility until the researcher is satisfied that you are fit to leave, or an emergency contact has been called to collect you. All experimental trials will be supervised by first aid qualified personnel. It is possible during the collection of venous blood samples, participants could experience discomfort and potential bruising at the site of collection, the discomfort would not be greater than venous blood samples a participant may have provided for past medical reasons (i.e. standard medical blood test). Nevertheless, if a participant does experience pain from this procedure, the researcher will immediately attend to the area with compression and ice.

Distribution of Results
All of your test results will remain confidential and will be coded, with the code kept separated from your personal details. Results will be electronically stored on a password protected computer hard-drive of the primary researcher. Only the researchers involved in this study will have access to data identifying you by name.

Alternative Uses of Data
All data collected during this study will be stored for the duration of five years at which time, data will be destroyed or electronically erased as necessary. During this period, data collected from this study may be used for scientific purposes other than those outlined above. At no time will private or identifiable participant information be used. Any data provided for alternative use will be coded as to eliminate the ability to identify the participant.

Questions If you would like to discuss any aspect of this study, please feel free to contact the following investigators: Dr James Fell, James.Fell@utas.edu.au, +61 3 6324 5485 Dr Cecilia Shing, Cecilia.Shing@utas.edu.au, +61 3 6324 5484 Dr Chris Abbiss, c.abbiss@ecu.edu.au , +61 (8) 6304 5740 Dr Jeremiah Peiffer, J.Peiffer@murdoch.edu.au, +61 8 9360 7603 Ms Emma Zadow, Emma.Zadow@utas.edu.au, 0430 417 295

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote

Appendix B, iii: Informed Consent

UNIVERSITY of TASMANIA | FACULTY OF HEALTH

Informed Consent

Does Time of Day Influence Pacing Selection in a 4 km Time Trial?

The investigators conducting this research project abide by the principles governing the ethical conduct of research and, at all times, undertake to protect the interest, comfort and safety of all athletes.

This form and accompanying Participant Information Document have been given to you for your own protection. They outline the experimental procedures and explain possible risks.

Your signature on this form will indicate that you:

1. Agree voluntarily to take part in this study
2. You have read the Participant Information Document provided and have been given a full explanation of the purpose of the study, the procedures involved and of what is expected of yourself as a participant. The researcher has answered all of your questions and has explained the possible problems that may arise as a result of your participation within this study
3. You understand that you are freely able to withdraw from the study at any time without needing to provide any reason.
4. You understand that you will not be identified in any publication arising out of this study.
5. You understand that your name and identity will be stored separately from the data, and these records are only accessible to the involved investigators. All data provided from yourself will be analysed anonymously using a code.
6. You understand that all data obtained during this study will be stored for five years during which your data may be used in retrospective research. The use of this data will be done so in an unidentifiable manner.
7. You understand that all personal information provided by yourself is treated as confidential and will not be released by the researcher to a third party, unless required to do so by law.

Any queries regarding the procedures and/ or rational used within this study are welcome at any time; please feel free to contact Dr Cecilia Shing or Ms Emma Zadow. If you are

dissatisfied with the response, you are encouraged to contact the Executive Officer of Tasmania on 6226 7479.

Participant

Name: _____

Signature: _____

Mobile No: _____ Email: _____

Date: ___/___/____

Investigator

Name: _____

Signature: _____

Mobile No: _____ Email: _____

Date: ___/ ___/ ____

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote

Appendix B, iv: "Does time of day and short-duration high-intensity exercise influence coagulation and fibrinolysis?" Conference Poster

UNIVERSITY of TASMANIA

Does time of day and short-duration high-intensity exercise influence coagulation and fibrinolysis?

Emma K. Zadow[1], Cecilia M. Kitic[1], Sam S.X. Wu[1,2], James W. Fell[1] & Murray J. Adams[1,3]

[1]Sports Performance Optimisation Research Team, University of Tasmania, [2]Department of Health and Medical Sciences, Swinburne University of Technology, [3]School of Veterinary and Life Sciences, Murdoch University

Introduction

- Exercise has considerable effects upon haemostasis, with activation dependent upon the duration and intensity of the exercise bout [1].
- Markers of coagulation and fibrinolysis have been shown to possess circadian rhythms, peaking between 0600-1200 h [2,3].
- The time of day exercise is performed may influence haemostatic activation.
- **This study aimed to examine coagulation and fibrinolytic response to short-duration high-intensity exercise when completed at different times of the day**.

Methods

- Fifteen male cyclists (VO_{2max}: 60.3±8.1 ml.kg^{-1}.min^{-1}) completed a 4 km cycling time trial (TT) on five separate occasions at 0830, 1130, 1430, 1730 and 2030 h.
- Venous blood samples were obtained pre- and immediately post-exercise, and analysed for tissue factor (TF), tissue factor pathway inhibitor (TFPI), thrombin antithrombin complex (TAT) and D-Dimer using enzyme-linked immunosorbent assays (Abcam, Melbourne, Australia).

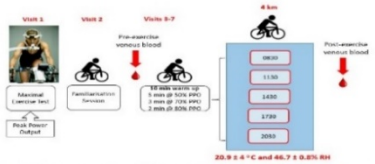

Figure 1. Study design: An initial maximal exercise test was completed followed by a familiarisation session, on separate days. In a randomised and counterbalanced order, cyclists completed a 4 km cycling TT, completed on their own bicycles. Pre-exercise blood samples were collected prior to a standardised warm-up after ten mins of supine rest, with post-exercise blood samples obtained immediately post-exercise.

Results

- Exercise ↑ TF, TFPI, TAT & D-Dimer (all p<0.002) (**Figure 2**).
- Pre-exercise TF & TFPI displayed a time of day response, with 0830 > 1730 h (p<0.001), and 1730 < 2030 h (p=0.008), respectively (**Figure 2**).
- No time of day effect was observed for TAT & D-Dimer (p>0.05) (**Figure 2**).
- No differences between TTs was observed for power output, TT time & heart rate (p>0.05) (**Figure 3**).
- A time of day effect for $\%VO_{2max}$ was observed, with 1730 h > 2030 h (p=0.04) (**Figure 3**).

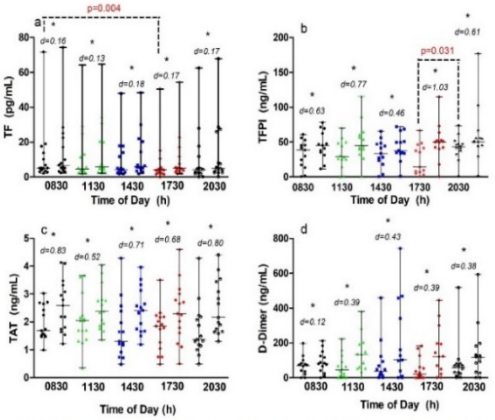

Figure 2. *Median (range) for pre- and post-exercise measures of a) TAT, b) TF, c) TFPI, and d) D-Dimer at 0830, 1130, 1430, 1730 and 2030 h. * significant difference from pre-post.* ***d****=effect size of pre to post difference.*

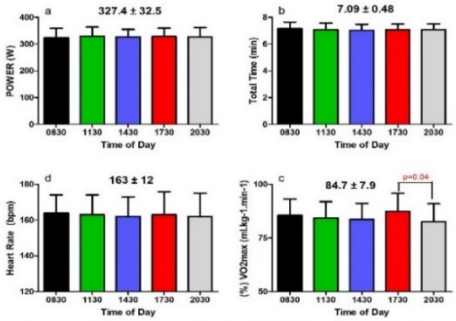

Figure 3. *Mean (± SD) for a) power (W), b) TT time (min), c) heart rate (bpm) and d) $\%VO_{2max}$ (ml.kg^{-1}.min^{-1}) measured during each 4 km TT at 0830-2030 h.*

Conclusion

- A short-duration high-intensity bout of exercise results in acute activation of coagulation & fibrinolysis, regardless of the time of day.
- With TF peaking at 0830 h, caution should be applied when prescribing a 4 km TT within the morning, especially in populations predisposed to hypercoagulability.

References

1. El-Sayed, M.S., et al. Arterioscler Thromb Vasc Biol, 2005. **25**(3): p. 646-9.
2. Pinotti, M., et al., Arterioscler Thromb Vasc Biol, 2005. **25**(3): p.646-9.
3. Kapiotis, S., et al., Circulation, 1997. **96**(1): p.19-21.

Contact Us
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SWINBURNE UNIVERSITY OF TECHNOLOGY

Murdoch UNIVERSITY

UTAS

Appendix C, i: Participant Information Sheet

Project Title: The effect of travel and marathons on haemostasis

Investigators A/Prof James Fell
Dr Murray Adams
Ms Nerolie Bost
Dr Shona Halson
Dr Cecilia Shing
Ms Emma Zadow
Dr Amy Johnston
A/Prof Julia Crilly
Dr Andrew Bulmer
Dr Indu Singh

Location School of Health Sciences
Locked Bag 1322
University of Tasmania
Launceston, Tasmania 7250

Contact Miss Emma Zadow

Emma.Zadow@utas.edu.au

Invitation

You are invited to participate in this study as you are:

1. Competing in the 2016 Gold Coast Marathon

Introduction

While exercise has been shown to be beneficial for overall health and wellbeing, it has been well reported that the duration and intensity of the exercise itself may lead to a transient increase in the activation of the coagulation system, resulting in an increased risk for thrombosis (i.e. blood clot) development. Furthermore, prolonged travel is also implicated with increased risk of blood clot development and athletes often travel long distances to compete in major events such as the Gold Coast Marathon. Such clots can be potentially fatal and avoidance is the safest option. However, there has been minimal research to investigate the interaction between prolonged travel and prolonged exercise, such as a marathon, on the markers associated with an increased risk of blot clots.

Several clothing companies that produce sports compression garments make claims that the compression clothing can also be of benefit for reducing blood clot risks associated with travel. It may be that wearing compression garments, such as compression socks, during a marathon, or in the post-race period may be of benefit for reducing the blood markers associated with increased risk of blood clots. However, there has been limited research to investigate these claims.

Participants

We require 80 healthy marathon runners competing in the 2016 Gold Coast Marathon. You need to be in good health with no major injuries or illness, known blood clotting disorders, or taking any anticoagulant medication such as aspirin, heparin, warfarin etc. As a possible participant in this study, you will be asked to visit a testing location on three occasions (24 hours before, immediately after and 24 hours after the race). Each visit would require approximately 15 minutes to complete for a total of 45 minutes.

During your initial visit, you will complete a health-screening questionnaire and provide information about your training, general health and travel to the event. You will then provide a small sample of blood taken from a vein in the front area of your elbow region. Some participants will then be provided with compression socks to wear during the race.

After the race, you will need to attend the testing location as soon as possible to provide another blood sample. You will also complete a questionnaire that asks questions about your race, such as your performance, effort, and any illness or injury. If you are treated for a medical event either during or after the race information will be collected about the nature of the medical event. Some participants will then be provided with compression socks to wear for the next 24 hours.

The final visit will occur approximately 24 hours after you finished the race. At this visit, a final blood sample will be collected and you will complete another brief questionnaire about how you have recovered and an indication of food, fluid and activity levels over the past 24 hours.

A pair of compression socks will be given to you during this visit if you have not previously received any.

Blood Collection

Throughout this study, you will be asked to provide three venous blood samples (15-20ml equal to about 3-4 teaspoons). These samples will be analysed for markers associated with increased risk of blood clotting and inflammation, muscle damage, protein and electrolyte levels.

Voluntary Participation

It is important that you understand that your involvement in this study is voluntary and we will respect your right to decline. There will be no consequences to you if you decide not to participate, and this will not affect your treatment/ service. If you decide to discontinue participation at any time, you may do so without providing an explanation. If you withdraw, all information you have provided will be destroyed. All information will be treated in a confidential manner, and your name will not be used in any publication arising out of the research. All of the research will be kept on a password-protected computer of the primary researcher.

Possible Benefits

Participants who complete this study will be provided a report of the key findings via email (if they agree to provide their email address). The findings of the study will identify potential risk factors for blood clots in athletes and whether strategies to reduce the risk, such as compression clothing before or after a race, are effective.

Possible Risks

It is possible during the collection of venous blood samples, participants could experience discomfort and potential bruising at the site of collection, the discomfort would not be greater than venous blood samples a participant may have provided for past medical reasons (i.e. standard medical blood test). Nevertheless, if a participant does experience undue pain from this procedure, the researcher will immediately attend to the area with compression and ice.

Distribution of Results

All of your test results will remain confidential and will be coded, with the code kept separated from your personal details. Results will be electronically stored on a password protected computer hard-drive of the primary researcher. Only the researchers involved in this study will have access to data identifying you by name.

Alternative Uses of Data

All data collected during this study will be stored for a minimum of five years. During this period, data collected from this study may be used for scientific purposes other than those outlined above. At no time will private or identifiable participant information be used. Any data provided for alternative use will be coded as to eliminate the ability to identify the participant.

Questions

If you would like to discuss any aspect of this study, please feel free to contact the following investigators:

A/Prof James Fell, James.Fell@utas.edu.au, +61 3 6324 5485
Dr Murray Adams, Murray.Adams@utas.edu.au, +61 3 6324 5483
Ms Nerolie Bost, nerolie.bost@health.qld.gov.au, +61 7 5687 5273
Dr Cecilia Kitic, Cecilia.Kitic@utas.edu.au, +61 3 6324 5484
Ms Emma Zadow, Emma.Zadow@utas.edu.au, 04
Dr Shona Halson, Shona.Halson@ausport.gov.au
Dr Andrew Bulmer a.bulmer@griffith.edu.au
Dr Indu Singh i.singh@griffith.edu.au
Dr Amy Johnston, amy.johnston@health.qld.gov.au
A/Prof Julia Crilly, julia.crilly@health.qld.gov.au

This study has been reviewed and approved by the Gold Coast Hospital and Health Service (GCHHS) Human Research Ethics Committee, Tasmanian Health and Medical Human Research Committee and Griffith University Human Research Ethics Committee. Should you wish to discuss the study with someone not directly involved, in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make an independent complaint, you can contact the Chairperson of GCHHS Human Research Ethics Committee on Ph.: + 61 7 5687 3879 or email: GCHEthics@health.qld.gov.au

You will need to quote ethics reference number **HREC/16/QGC/48**

Appendix C, ii: *Study Timeline*

Saturday 2nd July: 10:00 am to 2:00 pm:

- Please report to the registration area located at the Gold Coast Convention and Exhibition Centre (Gold Coast Highway, Broadbeach), Office 3 on level 1, between Hall 3 and 4 (**Figure 1**).
- We will collect the following information from you:
  1. Informed Consent
  2. Medical history and Questionnaire
  3. Height and Weight
  4. First blood sample
- You will also be randomly allocated to either the compression or non- compression sock group, with the compression socks to be worn during the marathon.
- You will be provided with a wristband (**below image**) to be worn during the marathon. This is so we can identify you as registered participants within our study and so we can direct you to the medical tent in order to collect your post marathon blood samples.

Sunday 3rd July: Marathon Day (within 1-hour post marathon completion)

- If you have been assigned to the compression sock group, please wear these throughout your marathon run ☺

Please ensure you have placed your wristband on.

- A helper will be located at the finish line of the marathon and will direct you to the medical tent (**Figure 2**), where we will:
  1. collect your second blood sample
  2. Ask you to complete a post exercise questionnaire
- You will then be randomly allocated to either the compression or non-compression sock group (with compression socks provided) to be worn throughout your recovery (24 hours post marathon), prior to your final blood sample being collected.

Monday 4th July: 10:00 am to 2:00 pm

- Please report to the medical tent (where your blood samples were collected immediately after the marathon) to complete the following:
  1. Provide your third and final blood sample
  2. Complete a follow up questionnaire
- If you have not been provided with compression socks at this stage, you will then be provided with some throughout this visit.

Appendix C, iii: Participant Recruitment Questionnaire

Demographic Information:

Athlete Name:

Age:

Sex: M F

Height: _____Weight: _____

Describe your current physical activity/ exercise levels:

1. Frequency (average sessions per week) _____
2. Duration (average minutes per session) _____

Have you trained/ competed using compression garments before?

YES / NO

If yes, I use it for (tick all applicable): training/recovery/sleep/travel/other

Brand (i.e. 2XU, skins): _____
Type of compression (i.e. socks, leggings, sleeves):

Gold Coast Marathon Details:

1. Are you registered to complete the marathon? Y / N
2. Registration/ bib number:
3. What is your expected finishing time for the Gold Coast Marathon: ____(h) _____(m)

Travel Information:

4. What will be your primary mode of transport to the event?

   Airplane **Car/ Bus/Train** **Boat**

5. Which state of Australia or country from outside Australia are you travelling from to participate in the Gold Coast marathon?

6. What is your expected travel time? (i.e. 3 hours).

   Travel time: ____ (h): _____ (m) Breaks between travel: _____

7. When are you departing the location (Q3)?

   Date and time: _____

8. When are you arriving at the Gold Coast?

   Date: _____

   Time: _____

9. When are you leaving/ departing the Gold Coast?

   Date/ time: _____

10. Did you wear or do you intend to wear compression garments when travelling to the Gold Coast?

YES / NO

General Health and Medical Screening:

11. Are you currently in good health with no major injuries or illnesses?

YES / NO

Further information:

12. Are you on the oral contraceptive pill?

YES / NO

13. Do you have any blood clotting disorders or metabolic conditions? (i.e. have Cardiovascular disease or Diabetes)

YES / NO

Further information:

14. Are you on any anti-coagulation/ blood thinning medications? (i.e. aspirin, warfarin, heparin)

YES / NO

Further Information:

15. Do you have a family history of blood clotting disorders? (i.e. mother/ father, which clotting disorder they may have)

YES / NO

Further information:

16. Do you have a previous history of venous thrombosis? (i.e. deep vein thrombosis or pulmonary embolism).

YES / NO

Further Information:

17. Is there a family history of venous thrombosis? (i.e. mother/ father, age of thrombosis)

YES / NO

Further Information:

General:

18. Are you available to provide all three (pre marathon (+24 h), immediately post marathon and 24 h post marathon) required blood samples between 10 am and 2 pm?

YES / NO

Appendix C, iv: Informed Consent

Title of Project: The effect of travel and marathons on haemostasis

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The investigators conducting this research project abide by the principles governing the ethical conduct of research and, at all times, undertake to protect the interest, comfort and safety of all athletes.

This form and accompanying Participant Information Document have been *given* to you for your own protection. They outline the experimental procedures and explain possible risks.

Your signature on this form will indicate that:

- I acknowledge that the nature, purpose and contemplated effects of the project as far as it affects me, has been fully explained to my satisfaction by the research worker and my consent is given voluntarily.

- The details of the procedure proposed have also been explained to me, including the anticipated length of time it will take, the frequency with which the procedure will be performed, and an indication of any discomfort, which may be expected. I understand that my involvement requires:

    - Completion of questionnaires, providing a venous blood sample and, possibly, the wearing of compression socks.

- I understand that there are the following risks or possible discomfort:
    - Bruising or discomfort at the site of blood collection

- I have been informed that all information and results will remain confidential (disclosed only to myself) and the results of any tests involving me will not be published so as to reveal my identity.

- I understand that I am free to withdraw from the project at any stage and any of my data/specimens that have been collected. My withdrawal will not affect my legal rights, my medical care or my relationship with the hospital or my doctors.

- I understand that I will be given a signed copy of this patient information sheet and consent form. I am not giving up my legal rights by signing this consent form.

- I understand that the trial will be conducted in accordance with the latest versions of the *National Statement on Ethical Conduct in Human Research 2007* and applicable privacy laws.

Name of participant: _____

Signature of participant:_____ Date:_____

- I have explained this project and the implications of participation in it to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

Name of investigator: _____

Signature of investigator: _____ Date:_____

Appendix C, v: "Does wearing compression socks during a marathon influence coagulation and fibrinolytic activation?" Conference Poster

UNIVERSITY of TASMANIA

Does wearing compression socks during a marathon influence coagulation and fibrinolytic activation?

Emma K. Zadow, Murray J Adams, Sam S.X. Wu, Cecilia M. Kitic, Indu Singh, Avinash Kundur, Nerolie Bost, Amy N.B. Johnston, Julia Crilly, Andrew C. Bulmer, Shona L. Halson & James W. Fell

Introduction

- Compression socks are widely used for the prevention of lower extremity pathologies, (i.e. deep vein thrombosis)[1].
- Compression garments are frequently used by athletes due to the proposed enhancement of exercise performance & recovery [2].
- The impact of compression socks on haemostatic activation during endurance exercise has yet to be extensively investigated [3].
- **This study investigated the effect of compression socks on exercise-induced activation of coagulation and fibrinolysis following a marathon run.**

Methods

- Runners (43 males & 24 females) (**Table 1**) were randomly assigned to a compression (SOCK) or control (CONTROL) group & completed a 42.4 km marathon.
- Venous blood samples were obtained PRE (-24 h), and immediately post-marathon (POST), & analysed for thrombin antithrombin complex (TAT), tissue factor (TF), tissue factor pathway inhibitor (TFPI) & D-Dimer using enzyme-linked immunosorbent assays (Abcam, Melbourne, Australia)

Figure 1. Study design: *24 h pre-marathon, runners provided a pre-marathon blood sample, with 2XU compression socks (CS) provided to SOCK group (runners instructed to wear CS during the marathon ONLY). Marathon day- CS worn by SOCK group only, no CS worn in CONTROL group. POST-marathon blood sample collected immediately POST-marathon.*

Results

- SOCK attenuated the magnitude of change (PRE-POST) for D-Dimer vs CONTROL group (median (range) SOCK: +9.02, -0.34 to 60.7 ng/mL, CONTROL: +25.48, 0.95 to 73.24 ng/mL; p=0.008) (**Figure 1**).
- TF significantly ↑ POST-marathon in SOCK and CONTROL groups (median (range) SOCK: +1.19, -7.47 to 9.11 pg/mL, p=0.001, CONTROL: +3.47, -5.01 to 38.56, p=0.001) (**Figure 2**), no significant differences observed between SOCK & CONTROL groups.
- No significant POST-marathon changes observed for TAT & TFPI (p>0.05) (**Figure 2**).

Table 1. *Mean (± SD) baseline measures of physical and performance characteristics for the SOCK versus CONTROL participants.*

Variable	CONTROL (n=33)	SOCK (n=34)	P value
Age (years)	45.2 ± 11.5	43.5 ± 10.5	0.662
Finishing time (hour:min:secs)	4:27:26 ± 1:16:15	4:29:23 ± 1:17:19	0.106

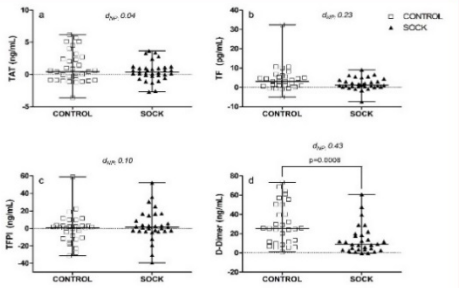

Figure 2. *Median (range) magnitude of change (Δ) between PRE-POST measures of a) TAT, b) TF, c) TFPI, and d) D-Dimer for the CONTROL and SOCK group.* d_{NP} = *effect size.*

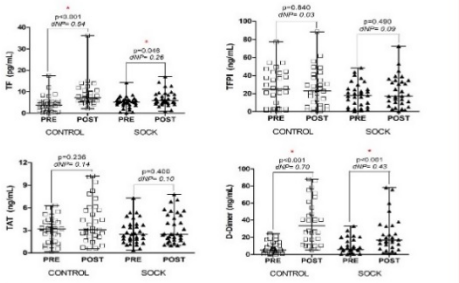

Figure 3. *TF, TFPI, TAT and D-Dimer for the CONTROL and SOCK group. Data are presented as Median (range).* d_{NP} = *effect size.*

Conclusion

- Compression socks may reduce exercise-associated haemostatic activation when completing prolonged strenuous exercise.
- Activation of coagulation & fibrinolysis was apparent in runners completing the marathon, regardless of wearing compression socks or not.
- Overall coagulation & fibrinolytic activation tended to be lower within runners wearing compression socks during the marathon.

Acknowledgements

- The authors would like to thank the runners for volunteering their time to complete this study, whilst also acknowledging funding provided by 2XU.

References

1. Agu, O., et al., Br J Surg, 1999. **86**(8282-90.; 2. Kraemer, W.J., et al., J Orthop Sports Phys Ther, 2001. **31**(6): p. 282-90; 3. Zaleski,A.L., et al., Phys Sportsmed, 2015. **43**(4): p. 336-41

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Murdoch UNIVERSITY

Appendix C, iv. European Journal of Applied Physiology manuscript acceptance letter

From: em.ejap.af523.5c3d51.a0d53cba@editorialmanager.com
To: Emma Zadow
Subject: EJAP-D-17-00941R2: Your manuscript entitled Compression socks and the effects on coagulation and fibrinolytic activation during marathon running
Date: Saturday, 30 June 2018 9:39:58 PM

Ref.:
Ms. No. EJAP-D-17-00941R2
Compression socks and the effects on coagulation and fibrinolytic activation during marathon running
European Journal of Applied Physiology

Dear Ms Zadow,

We are pleased to tell you that your manuscript has now been accepted for publication in European Journal of Applied Physiology.

We are pleased that you chose our journal, and we look forward to receiving future submissions from you.

Best wishes,

Fabio Fischetti, M.D.
Editor
European Journal of Applied Physiology

Appendix D, i: European Journal of Sports Science manuscript acceptance letter

From: European Journal of Sports Science
To: Emma Zadow; emma_zadow@hotmail.com
Subject: European Journal of Sport Science - Decision on Manuscript ID TEJS-2017-0809.R2
Date: Saturday, 16 December 2017 9:06:43 AM

15-Dec-2017

Dear Ms Zadow:

Ref: Time of day and short-duration high-intensity exercise influences on coagulation and fibrinolysis

Our referees have now considered your paper and have recommended publication in European Journal of Sport Science.

We are pleased to accept your paper in its current form and this will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter, along with those of the editor who coordinated the review of your paper.

You will receive proofs for checking, and instructions for transfer of copyright in due course. The publisher requests that proofs are checked and returned within 48 hours of receipt.

Thank you for your contribution to European Journal of Sport Science. We look forward to receiving further submissions from you.

Sincerely,

Gregory Bogdanis, PhD, FECSS
Section Editor, European Journal of Sport Science
gbogdanis@phed.uoa.gr